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THE INDUCED SUSCEPTIBILITY OF THE GUINEA-PIG TO THE TOXIC ACTION OF THE BLOOD SERUM OF THE HORSE.[1]

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The tests and experiments on which this paper is based were commenced in April, 1903, in connection with the routine of diphtheria antitoxin production. It was customary at that time to inject samples of the serum into animals before putting it on the market in order to guard against any possible contamination with pathogenic bacteria. It was found that when guinea-pigs which had survived an injection with a mixture of diphtheria toxin and antitoxin were used for this purpose, they frequently died within a few hours. For guinea-pigs that had not been used before, the injection was nearly always harmless. During the past year there has been available a considerable material which could be used to extend these observations. This material, together with the data of the earlier tests, was put at my disposal by the director of the laboratory, Dr. Theobald Smith. The preliminary observations were made by him, and I gratefully acknowledge my indebtedness to him for valuable advice throughout the course of my own work in this field.

The phenomena with which this paper concerns itself belong to a class some members of which have long been known, and to which a number of examples have been added within recent years and months. Known to the French as "Anaphylaxis," to the Germans as "Ueber-emfindlichkeit" and to the English as "Hypersensitiveness," or more recently "Supersensitiveness," the class has certain reactions in common. These depend on the fact that certain substances acting on animals continuously or repeatedly, and in suitable dose, render the animal after a time, not immune, but more than normally susceptible to their further toxic action. The substances are unknown in the chemical sense. They are constantly associated with certain mixtures of albuminous substances, bacteria, or bacterial products and are recognized biologically by their specific reaction. An example generally known is the abnormal reaction developed

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when an animal infected with tubercle bacilli is injected with the products of growth of the tubercle bacillus. Blood serum contains a substance, or substances, which under suitable conditions develop a reaction of this character. A number of years ago it was known that the blood serum of one animal was frequently toxic for animals of another species when administered by injection directly into the circulation, and the pathological effects of such toxic action were studied. Flexner(1) (1894) in the report of such a study says, "On the contrary, I found that animals that had withstood one dose of dog's serum would succumb to a second dose given after the lapse of some days, or weeks, even when this dose was sublethal for a control animal." This isolated observation was not developed further.

Richet is credited with an observation of similar import on eel serum, at about the same time. The blood serum of the horse is not toxic for the guinea-pig in the accepted sense of the word, and this may be the reason that the reaction with which we have to deal was not noticed earlier.

Acknowledging a verbal communication from Professor Smith to Professor Ehrlich in regard to the phenomenon, Otto(2), working under the latter, formally drew attention to the reaction which we are now particularly concerned with. He easily repeated the fundamental observation that while horse serum is not a poison for normal guinea-pigs, it causes sudden death or severe illness in animals treated previously with the toxin-antitoxin mixture. He showed that a period of ten days or over must elapse after the injection of the mixture before the serum becomes an active poison. He showed further that the phenomenon could be developed for the blood serum of animals other than the horse, but that it was essentially a specific reaction. That is, horse serum did not become a poison for a guinea pig previously treated with a mixture of toxin and an antitoxin derived from the goat. He developed the fact that normal serum is as effectual in killing animals as is antitoxic serum. He was able to demonstrate a reaction subsequent to a single small dose of normal serum (1/500 c.c.–1/200 c.c.) as a sensitizing treatment, but this reaction was never so severe as in the case of animals treated with the toxin-antitoxin mixture. That is, the animals when tested became very sick, but none died. He states in a footnote, however, that he was able to develop the maximum reaction by giving repeated small doses of horse serum without diphtheria toxin. That is, the toxin was eliminated as an essential factor in the development of the reaction. The animals that survived the reaction, Otto discovered, were immune to subsequent injections of serum. From this he concluded that the substance which killed was a "haptin" in the Ehrlich sense. The more recently developed facts in regard to the immunity indicate that it depends on a combination of reactions which is without well-known analogy.

Rosenau and Anderson(3) and later Anderson(4) by work begun independently confirmed many of these results. They extended their observations in various directions by testing the influence of heat, antiseptics, precipitants, etc., on the toxic substance, and by showing that the offispring of hypersensitive female guinea-pigs are hypersensitive also.

Both Otto and Rosenau and Anderson state positively that death in this manner is unaccompanied by pathological lesions.

Currie (5) writing of the antitoxin rashes in human beings includes the guinea-

pig reaction in his discussion. He reported no experiments with the guinea-pig, and his explanation of the mechanism of the reaction is more complicated than is necessary to explain the facts known in regard to the course of the reaction in the lower animal. Nicolle(6) working with the closely allied "Phenomenon of Arthus" obtained experimental results comparable to my own, and they will be discussed later. Besredka and Steinhardt(7) have experimented with the immunity which is developed when the hypersensitive animal is treated with a large but not fatal dose of serum. Their results touch my work at one point only, and will be referred to later.

Gay and Southard(8) were the first to point out in writing the fact that the intoxication of the hypersensitive animal is accompanied by very obvious anatomical lesions. These lesions in their gross aspects were clearly described in the earliest notes in our laboratory, but were completely overlooked by the earlier experimenters elsewhere. Gay and Southard have worked them out in great detail and the pathological histology of this intoxication is the sound advance made by their work. These writers also formulated a theory to explain the development of the reaction which I will discuss in some detail after reporting on my own work.

Rosenau and Anderson(9) have more recently published results of observations along the lines previously laid down. The advance which concerns us at present is the fact that the transmission of the hypersensitive condition from mother to offspring is ante-natal in its accomplishment. The milk of the mother as tested by experiments in which the nurses were changed is not an essential factor in the transmission. They are now led to the opinion, opposite to that which they formerly held and to that of Otto, that the toxin injected with the sensitizing dose does not affect the degree of the resulting sensitization. It is probable that their earlier opinion is better supported by experiment.

Vaughan and Wheeler(10) have worked on the hypersensitive reaction to egg-white. They hold that the sensitizing dose induces the formation of an entirely new ferment which first gradually disposes of the original proteid injected and then remains for a long time stored in the cells as a zymogen. This zymogen is capable of being activated by the second injection of proteid and of splitting it into a toxic and a non-toxic portion more rapidly than the toxic portion can be safely disposed of. They are able to separate by a process of chemical mutilation a toxic portion which cannot sensitize the animal, and a non-toxic residue which sensitizes against the whole egg-white. Their case differs in important respects from that of the hypersensitiveness against serum. For instance they are unable to demonstrate any newly-formed substance in the blood of the hypersensitive animal which is capable of passively sensitizing a fresh animal.

Otto(11) in a recent paper, which came to hand after the completion of my work, has demonstrated by passive transfer, a newly-formed antibody in the blood serum of the hypersensitive animal. He believes, as I do, that this antibody is distinct from the horse serum "rest" present in the same serum—the "anaphylactin" of Gay and Southard. He does not, however, distinguish the hypersensitive reaction developed in the fresh animal by the transfer of a small amount (.1 c.c. to 2.5 c.c.) of hypersensitive guinea-pig serum after the two weeks' incubation period, from the immediate reaction (twenty-four hours) that can be developed by the transfer of 10 c.c. to 15 c.c. of the same blood or blood

serum. He was able to demonstrate the antibody in the blood serum of the immune or refractory animal, and on this ground doubts the opinion of Bedreka and Steinhardt, that the immunization in this instance is really a desensitization or exhaustion of the antibody. If he had made the more decisive intracirculatory test to determine the full development of the refractory state, or had injected a considerable surplus of serum over the amount needed to enable the animal to withstand the further subcutaneous or intraperitoneal injection of horse serum, he would probably have been willing to agree to their view in its essential features. Otto further shows that the combination of hypersensitive antibody with horse serum has no power to divert to itself guinea-pig complement when tested by the method of Bordet and Gengou. He holds, on general grounds, that this reaction is the manifestation in the guinea-pig of the "phenomenon of Arthus."

It is my purpose to present as briefly as possible our early and more recent experiments, in so far as they show the early, general observations on serum anaphylaxis. In extending these observations my work has been carried on in large part with a view to answering certain specific questions. These questions may be briefly stated, and for purpose of presentation my report of results will be, in part, grouped around them under the following heads:

I. Are the various methods of inducing the hypersensitive state and of detecting it of equal value in the determination of the nature of the reaction and of the factors involved?

II. Synopsis of early experiments.

III. What are the facts in regard to the transmission of anaphylaxis from mother to offspring, and what light do these facts shed on the problem of direct or active sensitization?

IV. What is the mechanism of the acute hypersensitive reaction? More particularly, is it possible to transfer the hypersensitive condition from animal to animal with the blood or blood serum?

V. What are the facts in regard to the immunity which is developed when a hypersensitive animal is treated with a sublethal dose of horse serum and what is the nature of this immunity? Included with the discussion of this question is the rather detailed description of a type of serum hypersensitiveness unusual in the guinea-pig. The reaction is largely localized in the subcutaneous tissues, and results in necrosis.

VI. A more general discussion of the problem as a whole.

VII. Summary.

I. METHODS OF SENSITIZATION AND TEST.

The quantity of serum given at the second or test injection and the method of its administration are of primary importance in the study of the reaction. The results obtained by different workers and especially the interpretation of these results have been influenced in no small degree by the particular method of test chosen by them. In our early work and in that of Otto, the guinea-pigs were sensitized by treatment with non-fatal toxin-antitoxin mixtures. They were tested after four weeks or later with from three to six cubic centimeters of serum injected subcutaneously. Under the circumstances all of the animals proved hypersensitive and about fifty per cent. of the cases were fatal. The incubation period found to be necessary for a positive result was about two weeks, but very few of the animals were used so soon. As other workers came into the field they felt it desirable to push the work faster, and using this method of injection after incubation of from ten to fourteen days they could not get such consistent results. Rosenau and Anderson adopted the intraperitoneal method; Besredka and Steinhardt developed the intracranial injection. Gay and Southard used injections of serum alone to sensitize, and found that they were unable to kill their animal when testing by the subcutaneous method They adopted the intraperitoneal injection for routine work, and called attention to the very great sensitiveness of the animals to a test injection made directly into the circulation. I have not used the intracranial and intraperitoneal methods, and my statements in regard to them are based on the reports of others. I have recently used the direct injection into the circulation for special purposes. I find that the injection directly into the heart is the simplest procedure. After some practice it is quite certain to succeed, and it can be carried out rapidly. About two cubic centimeters of horse serum can be introduced into a normal guinea-pig of 230 grams weight without causing any symptoms. The method, the accidents incident to it, and the controls necessary, are fully described by Morgenroth(12). My technique differs from his in that I work without an assistant, tying the animal out firmly on a suitable board. I use a smoothly-working glass hypodermic syringe of capacity of two and a half cubic centimeters instead of his canula and detached syringe barrel.

Tested by this method after an incubation period of two weeks the certainly fatal dose of serum for animals sensitized by the toxin-antitoxin mixture is probably about 1/100 c.c.; 1/200 c.c. of serum will cause severe symptoms, and 1/150 c.c. will sometimes kill. Thus in testing for the degree of hypersensitiveness it is possible to inject about two hundred certainly fatal doses. By the intracranial method the certainly fatal dose is about 1/20 c.c. By the intraperitoneal route three cubic centimeters is almost certainly fatal. By the subcutaneous method it is probably impossible to reach the certainly fatal dose because of the impossibility of getting rapid absorption. As five or six cubic centimeters always develop a well-marked reaction, it is probable that from fifteen to twenty cubic centimeters, if absorbed at about the same rate, would be certainly fatal. It is impossible to use such an amount in practice. It is obvious that results obtained by one of these methods cannot be at once applied to the subject in general without most careful consideration of the values involved. In working with animals feebly hypersensitive the subcutaneous method would often show no result, while used on animals thoroughly sensitized differences in degree of reaction would be entirely masked by even 0.1 c.c. given into the circulation. For differences among animals that are all very sensitive the subcutaneous injection is capable of giving the most instructive results, unless the delicate methods are more carefully standardized quantitatively than has so far been done. To make an application of the above considerations to the problem in hand we may consider: (*a*) The incubation period; (*b*) the influence of toxin on serum sensitization.

(*a*) The incubation period is not to be considered as abruptly terminating at a given day. I have made an animal quite sick by the intracardiac injection of two cubic centimeters of serum on the sixth day after a toxin-antitoxin mixture. Those who have used the subcutaneous injection at twelve to fourteen days have not had consistent results, but by about three or four weeks the hypersensitiveness seems to reach its maximum. Holding the animals longer than this does not seem to increase the percentage of fatal cases. These rather meager facts make it appear that the antibody on which the reaction depends is produced gradually from a

time very soon after the sensitizing injection, and that the total effective quantity increases for a period of several weeks. About the sixth day, or perhaps somewhat earlier, it can be detected by the most delicate method.

(*b*) Otto published the results of a considerable number of attempts to sensitize his animals with small doses of serum alone. He found that the animals were made hypersensitive, but not to the same degree as when the serum was mixed with toxin. None died when tested by the subcutaneous method. Gay and Southard had the same experience and in order to accomplish their purpose settled on the intra-peritoneal test injection. Recently Rosenau and Anderson have published a table of results showing that toxin does not increase the sensitizing action of serum when combined with it. In these experiments they used the intraperitoneal test injection. It seems plain that the subcutaneous method of testing is the better suited to decide a point which concerns the maximum of sensitization, and I believe that the earlier opinion of Otto is the better supported by experiment.

Accepting the results of Otto, who showed that the animals which had survived the injection of diphtheria toxin alone were normal in their reaction to horse serum, I have used such animals in two groups of four each to determine what treatment, if any, with serum alone would develop the maximum grade of hypersensitiveness. As the accompanying table shows, I have confirmed Otto's observation which he did not report in detail, that repeated small doses of serum alone could do this.

The treatment with 1/1000 c.c. serum given three times on alternate days, with the injection ten days after the third treatment, was about as efficient as the treatment with the toxin-antitoxin mixture and slightly more efficient than a treatment with 1/2000 c.c. serum on ten successive days, with the test treatment ten days after the last injection. Either method is much more efficient than the single treatment with three cubic centimeters or over, or than the single treatment with 0.03 c.c. to 0.0025 c.c. serum, reported in detail by Otto.

TABLE I.—*Effect of Repeated Small Doses of Serum.*

Guinea-pig Number.	Sensitizing Treatment.	Interval Between Treatments.	Test Treatment.	Result of Test Treatment.	Remarks.
4704	1/2000 c.c. serum, 10 injections in 11 days. Total 1/200 c.c.	10 days after last injection, 21 days after first.	5 c.c. serum subcut.	Slight immediate symptoms; late induration.	Slight induration after first injection with 1/2000 c.c. serum.
4701	do	do	do	Same.	
4658	do	do	do	Same + small late ulcer.	
4689	do	do	do	Dead 2 hours.	
4699	1/1000 c.c. serum, 3 times in 6 days. Total 1/333 c.c.	10 days after last injection, 16 days after first.	do	Dead in night.	Slight induration after 1st injection with 1/1000 c.c. serum.
4722	do	do	do	Dead 4 hours.	Well marked swelling after 1st injection of 1/1000 c.c. serum.
4737	do	do	do	Slightly sick; late ulceration.	
4696	do	do	do	Very sick; late ulceration.	

II. SYNOPSIS OF EARLY EXPERIMENTS.

The records of the laboratory show that thirty-eight guinea-pigs bred from untreated mothers and themselves not treated were injected with doses of from three to five cubic centimeters of normal or antitoxic horse serum. Of these not one was noticeably affected. Examined at the end of twelve and twenty-four hours the subcutaneous tissues often show no palpable œdema or induration. The latter statement does not cover the full number of animals reported on, as such examinations were not always made. We have no contrary case, however. Our results are thus in accord with those of others in showing that practically horse serum is not a toxic substance for the normal guinea-pig. But neither is it an indifferent solution of substances which can be entirely eliminated from the subcutaneous tissues by processes which govern the removal of normal saline solution, for example. In several instances the injection of 1/1000 c.c. or 1/2000 c.c. of serum in two cubic centimeters of normal saline solution has given rise to a well-marked œdema at the end of twenty-four hours. Also, as can be demonstrated by suitable test-tube experiments, horse serum normally contained small amounts of ambocepter active between guinea-pig red blood corpuscles and the complement of guinea-pig serum. Certain of its constituents are probably always removed by complex processes similar in kind to those which prevail in the case of the hypersensitive animal, and the tissues may, under certain circumstances, be mildly injured in the course of their elimination.

Thirty-six animals treated with from two and a half to five cubic centimeters of serum as a first dose were subsequently once or twice injected with similar quantities. Of these thirty-three remained without symptom or lesion. One showed symptoms at the second treatment. One died at the second treatment, and one died at the third injection. The intervals between treatments have varied between thirteen days and four months, and have most frequently been between three and six weeks. The animals that died received their fatal treatment after an interval of sixteen days in each case. These results agree with those obtained elsewhere, and show that a single or repeated large dose of serum may render the animal susceptible to a subsequent similar dose, but that it is a

much less efficient way of inducing a hypersusceptibility. It is quite possible that the fatal results in the two cases may be due to the accidental absorption of serum directly into the circulation through a vessel injured by the injection. Or it may be that there is really a very great difference in the reaction of the individual animals to the first large dose. However that may be, our cases are evidently not comparable to those of Gay and Southard, who found that the large dose always rendered the animal hypersensitive if enough time were allowed to elapse before the test injection. As the intervals they report between the sensitizing and intoxicating injections were as a rule shorter than ours, these differences in result must also be attributed to a difference between the effects of the intraperitoneal and subcutaneous injections. In these experiments they used the intraperitoneal route for both first and second injections, and in this way avoided the great binding power which the subcutaneous tissues probably have for the toxic principle here involved.

Twenty-five guinea-pigs which had survived the treatment with a mixture of diphtheria toxin and antitoxin were injected with large doses of horse serum. In each instance in which the dose injected was one cubic centimeter or over the animal was made sick. Fourteen of them died. In our experience as well as in that of Otto and Rosenau and Anderson, it is a law without exception that treatment with a toxin-antitoxin mixture renders the animal susceptible to an acutely acting toxic substance in normal and antitoxic horse serum. The exact amount of serum injected with the mixture, if between 1/100 c.c. and 1/500 c.c., and the exact interval between the injections, if between two weeks and three months, *are indifferent matters* in the development of the reaction. The same may be said of the local lesion caused by the mixture, and of the genealogy of the animal.

The length of time that such an induced susceptibility may persist has not been fully determined so far. I have been able to test thirteen old females with intervals after sensitizing varying from eleven months to two years. Compared with those tested in less than four months after sensitization, these animals gave less reaction. Two of them, at eleven and sixteen months, respectively, injected with five cubic centimeters of serum gave no reaction.

Three animals of the series died, one after an interval of twenty-two months between sensitizing and test injections. The others ranged between these extremes. The reaction is thus a slowly disappearing one which may probably in individual instances persist throughout the life of the animal.

III. HYPERSENSITIVENESS TRANSMITTED FROM MOTHER TO OFFSPRING.

Our experience in injecting large doses of serum (from three to six cubic centimeters) subcutaneously into guinea-pigs bred from mothers that had been treated with the toxin-antitoxin mixture or with horse serum alone is shown in the following table.

TABLE II.—*Transmitted Hypersensitiveness.*

Treatment of Mother.	Number of Mothers.	Number of Offspring.	Result.
Toxin + Serum, 1/100 c.c. to 1/500 c.c.	27	41	Well 17 Dead 24
Serum only. Large dose, 3 c.c. to 5 c.c.	4	6	Well 2 Symptoms 2 Dead 2

The fact that the increased susceptibility generated in a female guinea-pig by treatment with a sublethal toxin-antitoxin mixture is transmitted to her offspring as first published by Anderson, is clearly shown. It is also seen that treating the mother with a single large dose of horse serum renders the offspring hypersensitive. Gay and Southard found that the offspring of their guinea-pigs sensitized with serum alone were hypersensitive in several instances But in our experience only a percentage of the animals bred from hypersensitive mothers are abnormal in their reaction. It might be supposed that the mothers of those young which do not react are not themselves hypersensitive. We were able to test this in one case and found that the mother still gave a moderate reaction, although her offspring at the time of test gave none. Furthermore, several females have given birth to individuals of each class, the normal and the hypersensitive. In one case an entire litter (four animals) of one female was tested on the same day with the same

dose of serum (six cubic centimeters subcutaneously). Two of them died within fifteen minutes, the other two gave no reaction. It is obvious that if the statement of Rosenau and Anderson, that the hypersensitive state is always transmitted from the mother to offspring (and by inference to all of them), be essentially true, the law must be limited in its application by certain conditions. To reason from the published experiments of the last mentioned authors, from our own experience, and from analogy with the other cases of transmitted immunity reactions, it seems that hypersensitiveness is by nature transmissible. But the mother probably transmits less effectively if her own initial sensitiveness is low and if the elapsed time between her sensitizing treatment and the birth of the offspring in question is long. The young animals seem to lose their sensitiveness with some rapidity, as they increase in age and size, and it is probable that the individual variation in the rate of this loss is considerable. It must be so if it is to account for the extreme difference between animals of the same litter tested on the same day. Alternatively one could assume an individual difference due to the influence of a normal father, but this would be without known analogy, and could only be justified by prolonged experiment.

The type of reaction obtained in these guinea-pigs which have acquired their increased susceptibility from the mother is interesting and calls for explanation. The animals rendered hypersensitive by treatment with the toxin-antitoxin mixture when treated with a subsequent injection of serum usually begin to show symptoms in about half an hour. Those that die usually do so in from two to four hours. Those that recover are ordinarily most ill at about four hours after the injection. From this time they recover rather rapidly, and are to all appearances well in from six to twelve hours. Occasionally death is delayed twelve hours and complete recovery to twenty-four hours. The animals tested for a transmitted susceptibility have reacted quite differently. Those that have proved hypersensitive have usually died in from fifteen to thirty minutes after injection. Death has occurred in five minutes. In two instances out of twenty-four death took place at the end of two hours; in one instance, in the night after some hours. The animals

that do not die show almost no reaction. They frequently brush the ears and nose with the forefeet and have a staring coat for half an hour, more or less. I have not seen a case in which an animal bred hypersensitive became severely sick and recovered. Rosenau and Anderson publish protocols which do not bear out this experience, but as they have used the intraperitoneal injection altogether the results are not strictly comparable. This sharp distinction between the reaction given by different young animals, extending as it does even to individuals of the same litter, together with the more rapid reaction given by the hypersensitive offspring, cannot perhaps be clearly explained by facts definitely proven at the present writing. Several factors may be mentioned as probably influencing the results. The young animal may be more easily injured by the ultimate toxic substance when it has the capacity to form or assimilate it. Absorption from the subcutaneous tissues in the young animal in so far as it depends on physical conditions, is probably more rapid than in the older one. The subcutaneous tissues of the animal sensitized by direct injection have probably been greatly influenced by the treatment, and in such a way that there is a local hypersensitiveness induced which leads to a local specific absorption of the toxic substance, tending to protect to a degree the cells of more vital organs. This will be rather definitely developed later. Such a local reaction may be less easily transferred from mother to offspring than a more general one depending on the conditions in the blood.

The fact that there is in connection with the phenomenon of serum hypersensitiveness a definite transmission of the susceptibility to the reaction from the mother, to her offspring, is at the present time very strong evidence for the proposition that the sensitizing injection causes the formation of an anti-body with which the second or test injection reacts.

IV. PASSIVE TRANSFER OF THE HYPERSENSITIVE CONDITION.

Gay and Southard attempted to determine the mechanism of the hypersensitive reaction. Their essential experiments from this point of view may be briefly restated. They found that the serum of hypersensitive guinea-pigs mixed with horse serum, incubated

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result
5038	27–VI–07. Serum of sensitive G. P. Inj. 1.75 c.c.; intracardiac.	1 hr.	27–VI–07. 1 hr. later. Inj. 1 c.c normal horse serum ; intracardiac.	No symptoms or very slight.
5035	27–VI–07. Ser. of sens. G. P. Inj. 3 c.c.; intraperitoneal.	1 d.	28–VI–07. Ser. nor. horse. Inj. 1 c.c.; intracardiac. 5 c.c. intraperitoneal.	Very slight symptoms.
5044	28–VI–07. Ser. of sens. G. P. Inj. 5 c.c.; intraperitoneal.	3 d.	1–VII–07. Ser. nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5047	28–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	6 d.	4–VII–07. Ser. nor. horse. Inj. 1 c.c.; intracardiac.	Slight symptoms.
5045	28–VI–07. Ser. of sens. G. P. Inj. 1.5 c.c.; intraperitoneal.	24 d.	24–VII–07. Inj. 1.1 c.c. ser. nor. horse; intracardiac.	Dead ½ hour.
5036	23–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	1 d.	24–VI–07. Ser. nor. horse. Inj. 6 c.c.; intraperitoneal.	Moderate but definite symptoms.
4975	2–VIII–07. Defibrinated blood of sens. G. P. Inj. 14 c.c.; intraperit. and subcut. 4–VIII–10 a. m. Same. Inj. 5 c.c.; intraperitoneal.	2 d.	4–VIII–07, 12 m. Inj. 1.5 c.c. nor. horse serum; intracardiac.	Severe symptoms; chloroform 4 hrs.; hemorrhagic lesions.
6010	Freshly defibrinated blood of sens. G. P. 8–VIII–07: Inj. 3 c.c.; intraperit. 9–VIII–07: " 6 c.c.; " 10–VIII–07: " 6 c.c.; " Total 15 c.c.	3 d.	11–VIII–07. Inj. 1.25 c.c. nor. horse serum; intracardiac.	Dead 3 minutes.
5079	Freshly defibrinated blood of sens. G. P. 25–VII–07: Inj. 3 c.c.; intraperit. 26–VII–07: " 5 c.c.; " 27–VII–07: " 2 c.c.; " 28–VII–07: " 5 c.c.; " Total 15 c.c.	4 d.	29–VII–07. Inj. 2 c.c. nor. horse serum; intracardiac.	Dead 4 minutes.

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*—Concluded.

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result.
6017	19–VIII–07. Freshly defibrinated blood of sens. G. P. Inj. 15 c.c.; intraperititoneal and subcutaneous.	1 d.	20–VIII–07. Serum of normal horse. Inj. 1.5 c.c.; intracardiac.	Dead 2 minutes.
6059	11–IX–07. Serum of sens. G. P. Inj. 7.5 c.c.; intraperitoneal.	1 d.	12–IX–07. Nor. horse ser. Inj. 1.5 c.c.; intracardiac.	Very severe symptoms.
6058	12–IX–07. Serum of sens. G. P. heated to 60° ½ hr. Inj. 7.5 c.c.; intraperitoneal.	1 d.	13–IX–07. Nor. horse serum. Inj. 1.5 c.c.; intracardiac.	Moderate symptoms.
		Control Experiments.		
6013	19–VIII–07. Freshly defibrinated blood of normal G. P. Inj. 15 c.c.; intraperitoneal.	1 d.	20–VIII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5082	23–VII–07. *Diph. Toxin* — .18 c.c. Antitoxic serum 1/100 c.c. +. Inj. subcutaneous slight lesion.	6 d.	29–VII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	Slight but rather definite symptoms.

Note. — All of the fresh guinea-pigs used in the above experiments except No. 4975 weighed from 230 to 250 grm. It is doubtful if larger animals would have reacted as well. The first five animals illustrate the ultimate sensitization by small doses of sensitive serum. The second control experiment was made for the purpose of showing that the cases in which the passive transfer of sensitive serum occupied several days do not reach the beginning of the effective incubation period for direct or active sensitization even when the latter is tested for by the intracardiac method.

and injected into the circulation of normal animals, provoked no reaction. On the basis of two such experiments they apparently drew the conclusion that the serum of the hypersensitive animal does not contain an anti-body for the toxic substance of horse serum. They further showed that 1.5 c.c. of serum of a sensitive animal injected into a "fresh" animal rendered it in turn hypersensitive after the usual incubation period of fifteen days. They also found that the blood of one sensitized and subsequently immunized animal transferred to another hypersensitive animal contains no demonstrable toxic substance. If, on the other hand, the blood of the refractory animal is transferred to a fresh animal it sensitizes it after the usual incubation period. Otto has recently published experiments showing that a fresh animal may be rendered hypersensitive within twenty-four hours by the injection of the blood serum of a hypersensitive guinea-pig. Bearing on these points I submit the following tabulated results of experiments on the passive transfer of the hypersensitive condition from animal to animal.

I have not thought it necessary to detail the history of the animals from which the sensitizing blood was drawn. They were all guinea-pigs that had been through the treatment with a toxin-antitoxin mixture some weeks previously. In order to eliminate a possible individual variation in the blood of different animals a mixture of bloods was usually employed. As it was unknown whether the intermediate substance was a labile body or not, and whether it was free in the serum or might not perhaps be bound to corpuscles, many of my attempts to transfer were made with freshly defibrinated blood including the corpuscles.

The results show definitely that there is in the defibrinated blood and in the blood serum of guinea-pigs hypersensitive to horse serum a substance which, when injected into normal guinea-pigs, renders them also hypersensitive to horse serum after a lapse of twenty-four hours.

The further study of the characteristics of this substance must be left to the future. One experiment shows that it is not destroyed by heating the serum to 60° C. for half an hour. Otto has found that it has no power to divert complement when combined with horse serum.

Gay and Southard's observation that a smaller quantity of sensitive or refractory serum can sensitize after the incubation period, I am able to confirm. I agree with them in the belief that this is a manifestation of a retained element of horse serum. But I think that this acts as an active sensitizer and is entirely distinct from the anti-body which takes part in the intoxication.

V. IMMUNITY OR ANTIANAPHYLAXIS.

It was very soon found that if a hypersensitive animal were injected with a large dose of serum but survived the reaction, a second large dose within a few days produced less reaction or none at all. Otto, who first recorded this observation, dismissed the matter in a sentence by assuming that the toxic substance is a haptin in the Ehrlich sense. It has since been found that the reaction is very different in its time relations, at least, from other immunity reactions. Twenty-four hours, or perhaps less, is all the time required to bring this immunity to its full protective force, as has been pointed out by Besredka and Steinhardt. The first of the following experiments is illustrative. Comparison of the first with the second experiment shows, too, that it is the quantity of serum injected and the point of injection that are important, rather than the fact that the animal has survived the reaction.

G. P. 4764. Sensitized Jan. 29, 1907.

$\frac{\text{Diph. Toxin .21 c.c.}}{\text{Serum 1/175 c.c.}}$ Small ulcer; paralysis.

March 28, 1907, 11 a. m. Serum of normal horse No. 93; 0.5 c.c. injected subcutaneously.

6 p. m. Quite sick.

March 29, 1907, 10 a. m. Well.

5.0 c.c. normal serum of horse No. 93; injected subcutaneously. No symptoms.

G. P. 5014. June 8, 1907. Sensitized.

$\frac{\text{Diph. Toxin .215 c.c.}}{\text{Serum 1/300 c.c.}}$ No lesion.

June 28, 1907: Normal serum of horse No. 106.

1/200 c.c. serum + 199/200 c.c. salt sol. by intracardiac injection. Severe symptoms; convulsions.

June 29, 0.1 c.c. ser. + 0.9 c.c. salt sol. by intracardiac injection. Dead after 3 minutes.

Besredka and Steinhardt have shown that it is possible to immunize in twenty-four hours against the more delicate intracranial injections and I have been able to extend this to the intracardiac injection.

The condition which makes it possible to reduce so rapidly the hypersensitiveness in the animal I believe to be a local hypersensitiveness of the subcutaneous tissues, which tends to hold the active substance of the serum at the point of injection, and so greatly to lessen its absorption rate. If the serum be so gradually introduced that this local reaction is effective, the anti-body on which the hypersensitive reaction depends may be entirely neutralized without killing the animal, or even rendering it appreciably sick, even though the test serum be introduced into the circulation. The following experiment demonstrates these points.

Three hypersensitive guinea-pigs[2] were treated as follows:

Sept. 19: 8 p. m.	0.5 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 8 a. m.	2.0 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 12 m.	5.0 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 8 p. m.	5.0 c.c.	Normal horse serum, intraperitoneally.	No symptoms.

September 21, 10 a. m. One of the animals received 1.5 c.c. normal horse serum by intracardiac injection; no symptoms. The other two animals were now bled, the blood was defibrinated and 15 c.c. was injected into the peritoneal cavity by a fresh normal guinea-pig weighing 240 grm. September 22, 4 p. m. After an interval of 30 hours this last animal received 1.75 c.c. normal horse serum by intracardiac injection; no symptoms.

This experiment is controlled by those on guinea-pigs Nos. 4975, 6010, 5079, 6017, 6058, 6059, of Table III. It shows conclusively that the substance on which the passive transfer of the hypersensitive reaction depends is removed from the circulation of the hypersensitive animal by the gradual introduction of large amounts of horse serum. The experiment could equally well have been considered in the section on the passive transfer of the hypersensitive state as showing that the anti-body there demonstrated was really a vital factor in the acute reaction.

As above stated, I believe that it is a local hypersensitiveness in the subcutaneous and peritoneal tissues which makes possible this

[2] These animals were the same which were used to obtain the serum to sensitize guinea-pigs Nos. 6058 and 6059. See Table III.

rapid neutralization of the anti-body without general symptoms. Under certain conditions which are not as yet fully determined this local hypersensitiveness may be greatly exaggerated. The animal is then fully protected against the acute intoxication, but its life is later sacrificed to the severe reaction in the subcutaneous tissues and abdominal organs. This type of reaction, well known in the rabbit, has not been observed heretofore in the guinea-pig, and I will, therefore, describe the cases which I have encountered in some detail.

In a number of instances I have departed for one or another reason from the usual preliminary or sensitizing treatment. In four cases I repeated, after a number of weeks, the original toxin-antitoxin mixture as nearly as might be. In twelve cases I fed serum by mouth to hypersensitive animals. In eight cases, reported above, repeated small doses of serum were given over a period of several days as a sensitizing treatment. It is not possible to discuss at present all of these experiments from the point of view from which they were undertaken. But the animals had one interesting feature in common when subsequently tested with five or six cubic centimeters of normal serum by the subcutaneous method. Several of the animals of each group died acutely with the usual symptoms. Three of the animals that received serum by mouth gave no reaction whatever. All of the remaining animals showed one or another phase of the following reaction. Acute symptoms following the injection were present or absent, but in all cases in which they were present the animal practically recovered from them in eight hours. At this time also the injected fluid was about absorbed, so that the subcutaneous tissues showed at most but a trace of thickening. From now on the animals became worse again. They became drowsy and had a staring coat with very watery eyes. Locally by the end of twenty-four hours after injection, there was a well-marked œdema, in some instances a very large one. In the milder cases the œdema was reabsorbed and the animal recovered in four or six days. In the severe cases the œdema became very large. Two animals died on the third day with a spreading œdema covering the whole abdominal and thoracic region. In the animals that lived beyond the third day, the œdema gradually became

harder, the overlying skin underwent a dry blackening necrosis, and finally the affected area sloughed out, leaving a bare ulcer varying with the severity of the case from one half to three inches in diameter. These ulcers were very slow to heal, the smaller ones taking a month, the larger ones three months from the time of injection to complete repair. These animals, as they have died, have been studied in gross and microscopically, and others have been chloroformed at one or another stage to complete the series. The results can be briefly stated.

The local lesion is at first an œdema of the subcutaneous tissues and abdominal muscles without cellular invasion. This is associated after several days with pronounced degeneration and necrotic changes in muscular tissues, connective tissues and skin. A dry superficial eschar overlying the œdematous subcutaneous tissues is formed. Through the breaks in the escharotic skin, bacteria gain an entrance. This infection developing on the fifth or sixth day calls forth a leucocytic reaction. The necrotic tissue is thrown off and leaves a bare ulcer, which, as has been said, heals very slowly. Internally one finds remains of the acute changes in the lungs and gastro-intestinal tract which have been so fully described by Gay and Southard. In harmony with the fact that the acute symptoms in these cases are slight, the lung lesions are always very small. The gastric and intestinal lesions are, on the other hand, very extensive. In two instances an irregular hemorrhagic ulceration occupying fully two thirds of the stomach wall was found. The lesion of the stomach has not been found in its stages of repair. In severe cases on the third day it is interesting to note that bacterial invasion has begun, and that a leucocytic reaction is only found at points where the bacteria have penetrated well within the necrotic gastric mucosa.

In the acute cases the lymphatic apparatus never displays definite pathological alteration, but in these cases of late reaction the spleen and mesenteric lymph nodes show interesting changes. The spleen frequently shows considerable areas of hemorrhagic necrosis. In one instance three-fourths of the organ was involved. Microscopically, the affected areas show extensive hemorrhage. The extravascular corpuscles and those in neighboring vessels are clumped,

fused, and often laked. The connective tissues and leucocytes in the affected areas are in various stages of degeneration. Where the necrotic areas border healthy tissue there is well-marked invasion of the hemorrhagic area by phagocytic endothelial cells. The healthy spleen tissue shows endothelial cell proliferation.

The mesenteric lymph nodes on the second and third day after injection show moderate œdema and congestion. The germinal areas show no alteration. The peripheral sinus and those at the hilum are dilated with serous fluid containing desquamated endothelial cells and red blood corpuscles in moderate number. In one instance a few threads of fibrin were found. The endothelial cells free in the sinuses are in stages of degeneration by lysis, and the red blood cells are agglutinated in clumps about them. The endothelial cells lining the sinuses are swollen, raised from the connective tissue cells backing them or in places are wanting altogether. In some instances the red blood corpuscles are clumped about cells that are still attached to the sinus wall. The bone marrow has been studied, but similar changes have not been found. These lesions of the lymphatic apparatus will receive more extended discussion in another paper.

Finally, I have paid particular attention to the condition of the blood in the vessels in all of the animals which I have been able to autopsy at the time of their death. The blood has frequently been drawn, suspended in salt solution and examined microscopically for evidence of agglutinative clumps. The vessels have been traced deep into the lungs in search for thrombi, and the sections have been carefully examined for the same. My conclusions are that fibrinous thrombi are never found and that such clumping of red blood cells as occurs is not enough to account for the lesions with which it is rather irregularly associated. The clumping as well as the hemorrhage are probably secondary to endothelial cell degeneration. The relation of this late reaction to the acute reaction will be further discussed with the theoretical considerations in a subsequent paragraph.

VI. GENERAL DISCUSSION OF PROBLEM.

I wish now to restate briefly the main facts in the case and to offer an explanation for them in so far as it seems possible to do so with our present knowledge.

The normal guinea-pig is not injured by the injection of normal or antitoxic horse serum into its body in any amount that the mechanical conditions at the site of inoculation will permit of. If, however, a normal guinea-pig be first treated with a small amount of normal horse serum and after a time be injected with a large quantity, it will become very sick or die. If it does not die it will recover rapidly. Within certain limits of quantity and time the larger the first or sensitizing treatment, the less injurious is the second test, or intoxicating injection. If a third dose, large or small, of horse serum be given twenty-four hours or more later, it is less apt to injure than the second dose, and this applies to all subsequent subcutaneous injections of horse serum. Female guinea-pigs which have been treated with horse serum one or more times whether themselves injured or not, transmit a hypersensitiveness to their offspring. The blood or blood serum of a hypersensitive animal if transferred by injection into a normal guinea-pig in a suitable dose, renders this animal also hypersensitive. This takes place within twenty-four hours if the dose be large enough, but with a dose that is ineffective at this time the sensitization can be accomplished after the same incubation period as that required for the injection of a small dose of horse serum to become effectual. Under various conditions, which all involve repeated treatments with small doses of serum before any large dose is given, the animals may be found hypersensitive in the usual way, or they may develop a more local reaction and die or recover after a longer time. The minor facts in the case will be referred to in the course of the following discussion.

The only attempt to explain comprehensively the mechanism of this particular reaction by one who has experimented with it is that of Gay and Southard. They take the view that the sensitizing substance in the horse serum is distinct from the toxic substance, but a critical study of their experiments does not reveal an adequate basis for this assumption, which in the absence of demonstrated facts in its support is unnecessary. They suggest that chemical analysis may support their view, but at present there seems neither more nor less reason for supposing that the toxic and sensitizing elements in horse serum are distinct substances than for assuming that diph-

theria toxin is really a mixture of a toxic substance responsible for its injurious effects and another substance which stimulates the production of anti-bodies. They have admittedly demonstrated by an experiment that is easily repeated, that the sensitizing principle of the horse serum remains for a very long time in the body of the animal into which it is injected, and that it can be transferred to the body of a second animal mechanically by the transfer of blood serum. That this substance acts as a sensitizer by irritating certain cells and increasing their affinity for the toxic substance (to use their terms) can be granted. But that is less definite than the statement that by injury it stimulates these cells to the production of an excess of receptors which are in part, at least, cast off and appear in the circulating blood as an anti-body. It is more probable that the offspring of hypersensitive female animals are hypersensitive because of the presence of this anti-body than because of the presence of the irritating serum constituent, as these workers suppose. On their supposition, the young should remain sensitive throughout their lifetime and transmit sensitiveness in favorable cases to the grandchildren. If experiment has not rigidly excluded these possibilities, it has at least rendered them very improbable.

There is at present, as I understand it, but one serious biological objection to the view that the toxic substance in small quantity is the sensitizing substance. The ultimate function of the anti-body produced must be assumed to be the elimination of the toxic substance. Why, then, since it is produced in such excess, does it not do this completely, very soon, and allow the hypersensitive condition to disappear? By supposing that the toxic substance was closely united to body cells at some stage of its elimination after uniting with the anti-body, either by extreme solubility in or close chemical affinity for certain of their constituents, one could understand the retention of a residual quantity sufficient to prolong the sensitive condition. But while there is no satisfying evidence against the unity of the substance in the serum, the work of Vaughan and Wheeler on egg-white has rendered it almost certain by analogy that the substance of the serum is chemically decomposed in the body of the animal so as to leave a non-toxic residue difficult of elimination, which keeps up the sensitization.

As I would state it provisionally, there is in horse serum a substance which is actually a mild and potentially a severe toxin for the somatic cells of the guinea-pig. It is actually a mild toxin because receptors to link the toxic substance to the cells are almost wanting. By the introduction of a minimal amount of this substance, the few receptors normally present are exhausted, and subsequently regenerated in great excess. They can now be transferred passively either from mother to offspring or mechanically with the blood serum. When the intoxicating dose of serum is injected a relatively large amount of the toxin can now be suddenly brought in contact with the susceptible cells and the acute reaction is developed. It must be remembered that this is a case in which the substance involved is not toxic for cells essential to the life of the animal when gradually administered, but only becomes so when it is suddenly introduced in excess.

There has been so far no opportunity to study the characteristics of this anti-body. It seems to be necessary to introduce the sensitive guinea-pig serum some time before injecting the horse serum if the reaction is to run at a rate that will injure the animal. This would indicate that the anti-body must be united to some constituent of the body cells before the horse serum is introduced if the animal is to be effectively sensitized. I have been able to show by one experiment that this antibody is not a very labile substance, but the details must be further developed.

It will be most instructive, perhaps, to consider for a time the hypersensitive animal as though it were a normal animal very susceptible to a particular toxin. If this animal is subjected to treatment with a considerable but not fatal dose of toxin it is subsequently found to have lost its susceptibility. The first thought, reasoning from analogy, is that it has acquired an immunity in the special sense of the term. The fact that this immunity is not transferred by the mother to her offspring and that the blood of such an animal has no protective value for an animal not immune, is not an argument against the animal itself being protected by anti-bodies against the toxin or some combination or reaction product of it. The cases in which such anti-bodies can be demonstrated by passive transfer while numerous do not even cover the whole field of immunity against bacteria.

But in this instance the facts in regard to the immunity, if it is well to apply the term to the condition, are adequately explained without assuming that such anti-bodies are formed.

Another well-known although not perfectly understood reaction by which a susceptible animal can be protected against a toxic or infectious agent is by an increase in the affinity of cells not essential to the life of the organism for the toxin. This may be manifested by the leucocytes in conjunction with a special class of anti-bodies, or by the subcutaneous tissues. The mechanism of the reaction of the latter tissues is not thoroughly worked out. In this instance I believe it probable that the late reaction with necrosis before described is an example of this form of protection. By modifying the sensitizing treatment the affinity of the subcutaneous tissues is probably raised to a point where they absorb and hold so much of the toxin that very little can reach the circulation and be carried to cells more vital to the life of the organism. If they are sufficiently hypersensitive they do this to their own destruction. This case is perfectly explained in this way if it be assumed that the effective receptors are retained within the cell, and that those in the circulation represent but an unessential fraction of the total.

Nicolle experimented further with the necrosis which Arthus first produced in the subcutaneous tissues of rabbits by repeated injections of horse serum. He found that following the early treatments there was developed an anti-body which when transferred passively with the blood serum to a fresh animal caused it to react to a first subcutaneous serum injection with necrosis. He has not tried the effect of intravenous injections on either the actively or passively sensitized rabbits, but it seems probable that the reaction is essentially the same as that in the guinea-pig, with the difference that the rabbit's subcutis is easier to sensitize to a point where it will protect the animal's life at its own expense. That the "Phenomenon of Arthus" is in its essential features identical with the "Theobald Smith Phenomenon" is the recently expressed opinion of Otto also. Neither is this the only intoxication in which the rabbit's subcutis exhibits a greater binding power for the poison than does that of the guinea-pig. Morgenroth(12) showed that this was the case for diphtheria toxin as well.

In the case of this particular reaction there is a third possible explanation for the failure of the animals to react for a period. Depending for its effect in large part on a rapid reaction rate, it can be understood that if the serum is introduced very slowly all of these receptors can be satisfied without injury to the animal. The hypersensitive animal then becomes neither immune nor refractory, but is for the time being normal, or rather is a normal animal with a recent large dose of serum. This result for the animal is the reverse of that which is brought about in the course of certain procedures in the immunization against bacteria. For a short time after a given treatment of a fresh or partially immunized animal, the animal may be more than normally susceptible to infection. This is supposed to be due to an exhaustion of the protecting receptors which are not yet sufficient in quantity to protect effectively against the dose administered and still leave a surplus. It is the same in this instance, except that as the anti-body or receptor is a detrimental rather than a protective agent, its removal is salutary. That the mere exhaustion of the abnormal receptors explains the immunity, or, as they term it, the antianaphylaxis, is the view of Besredka and Steinhardt, reasoning from the fact that the animals after a time become sensitive again. For these workers, however, the whole reaction takes place in the nervous system, while my impression is that the nerve cells take little part in the reaction except as they may be subjected to actual injury by the rapid exhibition of the toxic substance in the hypersensitive animal.

It is, perhaps, needless to emphasize the point that the explanation above offered is only intended to cover the facts in this particular reaction in so far as they have been experimentally developed. Other hypersensitive reactions seem to be more complicated, and more complex explanations have been offered for them. It would be unwise to impose such theories on the phenomenon here discussed in advance of the demonstration of facts requiring them.

A few words should be said in closing about the pathological anatomy of the serum intoxication. The work of Gay and Southard was instructive in showing the rapidity with which certain definite and important pathological alterations in tissue cells may be developed. My own studies also have shown conditions which are

interesting from the point of view of the specialist in pathological anatomy. But the significance of these changes in a general consideration of the subject remains doubtful. While Gay and Southard were able to show definite cellular lesions four minutes after injection, and while hemorrhages are not uncommon at that time, yet it is true that in the cases in which death occurs most quickly, lesions are much less frequent and widespread than in those in which it is delayed for several minutes or hours. It would probably be possible to kill animals in this way without demonstrable lesion. In most of the early cases, at least, I think the cause of death is rather to be referred to the disordered function of a single organ, or broken coordination between several organs than to anatomical lesion of any one organ or cell complex. Only pharmacological methods could show whether the action is on the nervous system. the heart, or the lungs directly, or on the heart and lungs through the nervous system. Anatomical studies show that whatever may be the immediate cause of death the toxic substance in its absorption, transmission and elimination injures to a greater or less degree cells of many types.

VII. SUMMARY.

Following the divisions before used, the results presented in the preceding pages may be briefly stated.

I. The particular method of sensitization and the place where the test injection is made have an important bearing on the results obtained by various workers. Comparing the results obtained by the various methods, we may conclude that the incubation period of the hypersensitive reaction is not sharply limited, but that there is a progressive increase in sensitiveness from the sixth day, and presumably before that, extending over a period of several weeks. It seems very probable that the degree of hypersensitiveness attained where the sensitizing dose consists of a mixture of diphtheria toxin and serum is greater than when a single dose of the same small quantity of serum is given alone.

II. Our early experiments, the first in this field, are in thorough agreement with those first reported by Otto, and shortly after him by Rosenau and Anderson.

III. This hypersensitive reaction is transmissible from mother to

offspring. The transmission is probably not equally effective in all cases, and individual young guinea-pigs probably vary greatly in the rate with which they lose their ability to react. As a result, not all of the young of a hypersensitive mother react to a subcutaneous dose of five cubic centimeters of serum given when they are four or five weeks old. The reaction in the young animals differs quite markedly from that in those actively sensitized. These differences are such as to indicate that in the mother there is a considerable localization of the reaction in tissues and organs whose destruction does not cause sudden death. This local reaction is a protective factor and is not transmitted to the same degree as the factors involved in the fatal acute reaction.

IV. The hypersensitive reaction to horse serum depends on the development of a special anti-body during the incubation period, which anti-body may be passively transferred to a fresh animal. If the dose of hypersensitive serum be sufficient, and the intoxicating injection be given directly into the circulation, this passive hypersensitiveness may be enough so that the animal will die when tested. There is also in the serum of hypersensitive guinea-pigs an uneliminated horse serum element or "rest," which is distinct from this antibody, and probably without influence on the course of the acute reaction.

V. The anti-body on which the hypersensitive reaction depends may be entirely neutralized by horse serum without causing symptoms. The gradual introduction of increasing doses over a total period of twenty-four hours suffices for this. The animal is then, properly speaking, neither immune nor refractory, but is essentially in the condition of a normal animal which has recently had a large dose of horse serum. This rapid neutralization is made possible by the great binding power which the subcutaneous and other relatively unimportant tissues have for the toxic element of the serum. The so-called "Phenomenon of Arthus" is probably the same reaction for the rabbit that we have here dealt with in the guinea-pig. The fact that the manifestation is more prominently a local one depends on racial differences. I have encountered cases in the guinea-pig in which the conditions in the rabbit are closely simulated.

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2. Otto, Leuthold-Gedenkschrift, 1906, i, pt. 1, 153.
3. Rosenau and Anderson, U. S. Marine Hospital Service, Hygienic Lab., 1906, Bulletin 29.
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6. Nicolle, *Annales de l'Inst. Pasteur,* 1907, xxi, 128.
7. Besredka and Steinhardt, *Annales de l'Inst. Pasteur,* 1907, xxi, 117, 384.
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9. Rosenau and Anderson, U. S. Marine Hospital Service, Hygienic Lab., 1907, Bulletin 36.
10. Vaughan and Wheeler, *Jour. of Infect. Diseases,* 1907, iv, 476.
11. Otto, *Münch. med. Woch.,* 1907, liv, 1665.
12. Morgenroth, *Zeit. f. Hygiene,* 1904, xlviii, 177.

ON THE INHIBITORY INFLUENCE OF EOSIN UPON SPORULATION.[1]

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Sporulation requires optimum temperature and suitable nutrient media. It is greatly influenced by various physical and chemical agents. Thus, in the case of Bacillus anthracis, the most studied in this respect of all spore-bearing organisms, no sporulation takes place at temperatures above 42° C.[2] or below 14° C.[3] Weil[4] places the lowest temperature at which sporulation takes place at 7° C. The presence of oxygen seems to be essential to the formation of anthrax spores.[5] Persistence of conditions unfavorable to sporulation through many successive generations of the organism gives rise to asporogenous strains in which virulence is often found greatly reduced or even totally absent.

Phisalix[6] succeeded in obtaining an asporogenous stain of B. anthracis by cultivating it at 42° C. for twelve successive generations. Roux[7] induced a similar biological alteration by means of a medium containing certain chemicals. Potassium bichromate in the ratio of 1 to 2000, or phenol in from 2 to 6 to 10.000 added to ordinary bouillon stops sporulation completely. Behring[8] found that various acids, alkalies, salts and certain antiseptics when used in suitable concentrations prevent sporulation. The antisporulative property of certain dyes was also described by Behring,[9] who states that safranin in 1 to 30,000, and methylene violet, cyanid, and malachite green in 1 to 200,000 to 1 to 600,000 exert a powerful restraining influence upon the growth and sporulation of B. anthracis. After

[1] Received for publication October 1, 1907.

[2] Phisalix, *Bull. méd.*, 1892, vi, 533.

[3] Kitasato, *Zeit. f. Hygiene*, 1890, viii, 198.

[4] Weil, *Zeit. f. Hygiene*, 1901, xxxvi, 451.

[5] Schreiber, *Cent. f. Bakt.*, 1896, xx, 353. Weil, *Arch. f. Hygiene*, 1901, xxxix, 205. Klett, *Zeit. f. Hygiene*, 1900, xxx, 420. Jacobitz, *Cent. f. Bakt.*, 1901, xxx, 232. Slupski, *Cent. f. Bakt.*, 1901, xxx, 396.

[6] Phisalix, *Bull. méd.*, 1892, vi, 533.

[7] Roux, *Annales de l'Inst. Pasteur*, 1890, iv, 25.

[8] Behring, *Zeit. f. Hygiene*, 1889, vi, 117.

[9] *Idem*, 1889, vii, 171.

two months' successive cultivation in the colored agar media, no permanent loss of the sporulating property resulted.

Schreiber[10] states that potassium phosphate of a concentration above 3 per cent. prevents sporulation of B. anthracis, B. subtilis and B. tumescens. Behring,[11] Bormans,[12] and Lecleff[13] found that B. anthracis does not form spores in blood serum, while Brieger, Kitasato and Wasserman[14] recorded a few instances in which B. tetani failed to sporulate in an aqueous extract of thymus gland.

While abundant work has been done with various spore-bearing aerobes, especially with B. anthracis, a similar study with anaerobic organisms has been so far neglected. In a recent study on the antitetanic property of certain dyes, Flexner and Noguchi[15] called attention to the fact that eosin in an adequate strength prevents the sporulation of B. tetani, the experimental details of which are given in a later paper by Noguchi.[16]

In the present communication I wish to present some of the results of experiments on the restraining influence of eosin upon the sporulation of various microbes. The varieties of bacteria subjected to experiment belonged to the aerobic and to the anaerobic organisms. Of the first, B. anthracis, B. megatherium, B. cereus, B. mesentericus, B. subtilis, B. ruminatus, and B. anthracoides, and of the second, B. tetani, B. anthracis symptomaticus, B. botulismus, B. œdema maligni, B. enteritidis sporogenes and B. putrificus were studied.

Eosin "Gelb" having been mixed with agar or bouillon in varying strengths, the inoculations of bacteria were made as usual. Stab and slant solid cultures were made. For the anaerobic bacteria, tissue-bouillon and a deep layer of glucose agar were employed. The agar tubes were incubated in an atmosphere of hydrogen. The results are tabulated.

Table I. indicates that the inhibitory action of eosin is most intense upon B. cereus and B. mesentericus, and least upon B. anthracoides, while B. subtilis, ruminatus, anthracis and megatherium occupy intermediary positions. All growth became uncertain when the concentration of eosin reached one per cent.; below this

[10] Schreiber, *Cent. f. Bakt.*, 1896, xx, 353.
[11] Behring, *Zeit. f. Hygiene*, 1889, vi, 117.
[12] Bormans, cited in *Baumgarten's Jahresberichte*, 1895, xi, 138.
[13] Lecleff, *La Cellule*, 1894, x, 349.
[14] Brieger, Kitasato and Wassermann, *Zeit. f. Hygiene*, 1892, xii, 137.
[15] Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 1.
[16] Noguchi, *Jour. of Exper. Med.*, 1907, ix, 281, 291.

TABLE I.—*Examination after 10 Days.*

Slant Agar with Eosin "Gelb."	B. cereus.	B. mesentericus.	B. subtilis.	B. ruminatus.	B. anthracoides.	B. anthracis.	B. megatherium.
Control (no eosin).	+ all	+ all	+ all	+ all	+ all	+ all	+ all
0.001 per cent.	+ few	+ few	+ many	+	+	+	+
0.01 "	—	—	+ few	+	+	+	+
0.05 "	—	—	—	+?	+	+?	+?
0.1 "	—	—	—	—	+	—	—
0.5 "	—	—	—	—	—	—	—
1 "	—	—	—	—	—	—	—
2 "	—	—	—	—	—	—	—

+ = spore formation.
— = no spore formation.
+? = sporulation doubtful.

strength, multiplication of the bacteria still takes place. These organisms, when cultivated in bouillon containing varying amounts of eosin "Gelb," appear to be even more sensitive to the action of the dye than when grown on a slant agar surface. But when the cultures are allowed to stand for many weeks, sporulation takes place in a medium containing as high concentration of the dye as 0.1 per cent. Concentrations above 0.3 per cent. prevent sporulation, to which effect is added restraint of growth and formation of chains of bacteria through imperfect multiplication. At times, marked degrees of involution occur. In no instances was growth discovered in bouillon containing two per cent. of the eosin.

In deep stab cultures the results were practically identical with those given in the table. Transplantation of the asporogenous bacilli into eosin-free media was associated with immediate return of the spore-bearing power. Many weeks' contact of the sporeless vegetative bacilli with the eosin exerted no enduring effect on the sporogenous property.

The influence of eosin "Gelb" on the sporulation of the anaerobic species has been shown in Tabie III. Recapitulated, they show that

TABLE II.

Bouillon Culture with Eosin "Gelb."	B. cereus.	B. mesentericus.	B subtilis.	B. ruminatus.	B. anthracoides.	B. anthracis.	B. megatherium.
Control (no eosin)	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.001 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.003 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. +	3 d. — 25 d. +
0.01 per cent.	3 d. — 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. + few	3 d. — 35 d. +
0.03 per cent.	3 d. — 38 d. +	3 d. + 38 d +	3 d. — 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. + few	3 d. — 25 d. +
0.1 per cent.	3 d. — 38 d. +	3 d. — 38 d. +	3 d. — } No surf. 38 d. + } membr.	3 d. — 38 d. +	3 d. — 38 d. +	3 d. — 25 d. + ?	3 d. — } No surf. 25 d. — } membr.
0.3 per cent.	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — } No surf. 38 d. + } membr.	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 25 d. — ?	3 d. — } No surf. 25 d. — } membr.
1 per cent.	No growth	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 25 d. —	No growth
	No growth	No growth.	No growth	No growth	No growth	No growth	No growth

d = day or days. + = spore formation. — = no spore formation.

TABLE III.—*Observations made after 29 Days on Certain Anaerobes Cultivated Aerobically in the Presence of Tissue.*

Bouillon Containing a Small Piece of Rabbit's Fresh Liver.	B. tetani.	B. anthracis symptomaticus.	B. botulismus.	B. oedema maligni.	B. enteritidis sporogenes.	B. putrificus.
Percentage of eosin "Gelb" in the bouillon						
Control (no eosin)	+	+	+	+	+	+
0.001 per cent.	+	+	+	+	+	+
0.003 per cent.	+	+	+	+	+	+
0.01 per cent.	—	+?	+	—?	—	—
0.03 per cent.	—	—	—	--	—	—
0.1 per cent.	—	—	—	—	—	—
0.3 per cent.	—	—	—	—	—	—
1 per cent.	No growth	No growth	No growth	No growth	No growth	No growth

+ = spore formation.
— = no spore formation.
? = spore formation doubtful.

the eosin in the strength of one per cent. completely inhibits all multiplication of the bacteria. The results are only very slightly different in the case of the cultivation of the anaerobic species freely in the air, in the presence of tissue (Table III.) and in an atmosphere in hydrogen in deep glucose agar. The phenomena of restraint are less pronounced in the latter media.

SUMMARY.

Sporulation of B. anthracis, B. subtilis, B. cereus, B. ruminatus, B. mesentericus, B. anthracoides and B. megatherium does not take place in an agar medium containing eosin "Gelb" in a concentration exceeding 0.5 per cent. In a concentration of 0.1 per cent. most of these bacteria fail to produce spores. The greatest sensitiveness is shown by B. cereus and B. mesentericus. In a bouillon medium sporulation is likewise inhibited by eosin, but after a longer time—seven weeks or more—sporulation still occurs where the concentration of the dye equals one-tenth per cent.

Sporulation of B. tetani, B. anthracis symptomaticus, B. botu-

lismus, B. œdema maligni, B. enteritidis sporogenes, and B. putrificus does not take place in a medium containing eosin "Gelb" in concentrations exceeding 0.03 per cent. With these organisms, no difference was noted in the final effect, depending on the medium employed. No permanent loss of power to produce spores ensues with the bacteria tested even after long sojourn in the eosinized media.

It may be stated that on the whole the inhibitory action of eosin was more pronounced upon the anaerobic than upon the aerobic species of bacteria employed in these experiments.

THE TRANSPLANTATION OF HUMAN CARCINOMATOUS MATERIAL INTO LOWER ANIMALS.*

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Although many attempts have been made to inoculate successfully the lower animals with portions of tumors from human beings they have in the great majority of cases been without results. In some instances the observers have claimed that they have been successful but their statements are, as a rule, not convincing.

Carl Lewin[1] discusses this question of transplantation at length and reviews the reports of those who have conducted such experiments. The following is a synopsis of the instances that he considers, separating them into two groups, according as the results of the transplantation were negative or apparently successful.

The first to be given are the negative instances.

Doutrelepont[2] experimented with the transplantation of human tissue into the lower animals with negative results.

Billroth[3] attempted to transfer human carcinoma and round cell sarcoma to dogs. No growth was however, obtained either by implantation or by injection into the jugular vein. Lebert and Wyss[4] injected a sarcoma emulsion into rabbits without any growth taking place. Fischl[5] injected rats intraperitoneally, intravenously and subcutaneously with a small cell sarcoma of the upper arm and with a melano-sarcoma of the lymph nodes. The tumors were absorbed without leaving the slightest trace. Duplay and Cazin[6] and Pawlowsky[7] transplanted sarcomatous tissue with no resulting growth. Roux and Metschnikoff[8] inoculated portions of a melano-sarcoma into the anterior chamber of the eye, and also beneath the skin of a young chimpanzee, with negative results.

* Received for publication October 14, 1907.

[1] Lewin, *Zeit. f. Krebsforsch,* 1906, iv, 55.

[2] Doutrelepont, *Virchow's Archiv,* 1869, xlv, 501.

[3] Billroth, *Wien. med. Woch.,* 1867, xvii, 437, 453.

[4] Lebert and Wyss, *Virchow's Archiv,* 1867, xl, 142, 532.

[5] Fischl, *Fortscritte der Medizin,* 1892, x, 159.

[6] Duplay and Cazin, *Semaine médicale,* 1892, xii, 61.

[7] Pawlowsky, *Virchow's Archiv,* 1893, cxxxiii, 464.

[8] Roux and Metschnikoff, *Bull. de l'Academie de med.,* 1903, s. 3, l, 101.

Many positive reports are given; they include the following.

Follin and Lebert[9] injected from sixty to seventy grammes of a mixture of mammary carcinoma and water into the jugular vein of a dog. At the end of fourteen days nodules about the size of a bean were found in the wall of the heart and tumors the size of a pin's head were found in the liver. No definite microscopic report is given.

O. Weber[10] injected portions of a carcinoma of the superior maxilla into the vena cruralis of a dog and also inoculated the same animal subcutaneously. On the sixth day granulations appeared at the site of the inoculation and continued growing until a mass the size of a man's fist was formed. As the granulation mass began to undergo necrosis the animal died. Similar granulation tissue was formed in a cat. Gouyon[11] obtained positive results from inoculation into dogs and guinea-pigs. Klencke[12] claims to have successfully inoculated horses and dogs with melanotic tumor cells.

Langenbeck[13] injected, on June 8, intravenously into a dog, cancer masses suspended in blood serum. On August 10 he killed the animal and found on the anterior surfaces of the upper lobes of both lungs two or three minute flat tumors that microscopically showed the structure of carcinoma. In the middle lobe of the left lung there was a hard mass about the size of a bean that also showed carcinomatous formation. Microscopically the growth consisted of a net-work of fine fibers between which were masses of closely packed cells. Lanz[14] injected a few drops of an emulsion of melano-sarcoma into the spleen of a dog. The animal became obviously cachectic and died at the end of one and a half months. A black mass at the site of the injection was found at autopsy. The subcutaneous tissue was slate-gray in color, the entire peritoneum dark, and the spleen deep black and soft. Pigment deposits were found in the liver, intestines, serosa, kidneys, lungs and epicardium. The pigment was generally of a deep black color, but in places it was greenish. It existed mostly free in the tissues but partially within the cells. Further transplantations were negative.

Lanz[15] also inoculated several rabbits with a peculiar tumor of the membranes of the central nervous system. After four months one animal showed a rapidly growing tumor in the left eye. After death the following conditions were observed: In the fatty tissue of the orbit there was found a stratified growth that had caused some necrosis of the neighboring bone. In the lungs and the kidneys and over the peritoneum, especially, were numerous soft tumors. Microscopically there was found "a parasitic infection of nearly all the endothelial cells of the peritoneum." The orbital tumor showed microscopically the structure of a sarcoma of the iris or choroid. The author believed that he had found in the parasite the cause of certain sarcomatous neoplasms.

[9] Follin and Lebert, *Virchow's Archiv*, 1867, xl, 538.

[10] Weber, Chirurgische Erfahrungen und Untersuchungen, Berlin, 1859, p. 289.

[11] Goujon, *Jahresbericht u. d. Leistungen d. gesamt. Medizin*, 1867, ii, pt. 1, 289.

[12] Klencke, *Archiv f. d. gesamt. Medizin*, 1843, iv, 484.

[13] Langenbeck, *Schmidt's Jahrbücher*, 1840, xxv, 99.

[14] Lanz, *Festscrift für Kocher*, 1891, p. 299.

[15] Lanz, *loc. cit.*

Jürgens[16] removed portions of *Sarcoma melanoticum carcinomatoides* thirty-six hours after death and implanted them into rabbits. In one animal he found at the end of eight days small tumors in the omentum and the mesentery. These had formed around the implanted tissue and consisted of large round cells and polymorphic cells. A second rabbit at the end of three weeks showed in the omentum two tumors about the size of a cherry stone. A third animal had small black tumors in the mesentery; and on the epicardium over the right heart some black soft structures about the size of a hemp seed.

Jürgens further reports the successful implantation of human sarcomatous tissue into rabbits. After intraperitoneal transplantation of a metastatic sarcoma of the brain there developed in the right eye of the rabbit a tumor the size of a hazel-nut of the same structure as the original growth. A second rabbit that had been inoculated in the eye with a melano-sarcoma, showed in the course of fourteen days, a tumor the size of a pea. He also succeeded in obtaining tumors in the lungs and intestines of a rabbit by inoculating it, twenty-four hours after its removal at autopsy, with a myxo-sarcoma of the ovary.

Mayet[17] reports that by injecting animals with the juice of malignant tumors he was able to obtain tumor growths. Von Bambeke[18] saw a tumor form in a rat five weeks after the transplantation of sarcomatous tissue. Reale[19] implanted subcutaneously a piece of a *Sarcoma cutaneum idiopathicum hemorragicum* (*Kaposi*) the size of a pea into a rabbit. At first the piece flattened down, but at the end of two years began to grow and attained the size of a chestnut; it was adherent to the adjacent tissues. The microscopic structure was that of an endothelioma or lympho-sarcoma and differed from the original material implanted. Reale considered the deviation in structure to be due to the influence of the new environment.

A. Vischer[20] in December, 1902, injected into the peritoneal cavities of a rabbit and a guinea-pig a cubic centimeter of a mixture of melanotic sarcoma and salt solution. On February 17, 1903, the rabbit was killed and in the peritoneal cavity there were found many small deeply pigmented nodules. The peritoneum did not show a trace of inflammatory changes. Microscopically the nodes consisted of a delicate connective tissue frame work with spindle cells and a few blood vessels. In the stroma were found many large round cells filled with pigment. Giant cells were also found. Nowhere were cells that resembled those of the tumor observed either free from pigment or poor in it. All those cells without pigment were either spindle or small round cells and had no resemblance to the cells of the tumor. The pigmented nodules were in all probability the result of reaction to the presence of foreign materials. Similar appearances can be obtained by the injection of india ink. The inoculated guinea-pig showed a condition similar to that in the rabbit.

[16] Jürgens, *Verhandl. der Berlin. med. Gesellschaft,* 1895, xxvi, 119. *Verhandl. der deutsch. Gesell. fur Chirurgie,* 1896, xxv, 84; 1897, xxvi, 154. Naturforscherversam. Düsseldorf. 1898.

[17] Mayet, *Lyon médicale,* 1902, xcviii, 17. *Gazette hebdomadaire,* 1902, vii, 64.

[18] v. Bambecke, *Semaine medicale,* 1893, xiii, 8.

[19] Reale, Tentativi d'inoculazione sperimentale del sarcoma cutaneo (tipo Kaposi). Napoli, 1902.

[20] A. Vischer, *Beiträge zur klin. Chirurgie,* 1904, xlii, 617.

Gaylord[21] inoculated dogs and guinea-pigs with carcinomatous tissue. In the dog there resulted numerous small nodules in the liver which were said to be cancerous. A guinea-pig showed carcinoma nodules in the lungs. Dagonet[22] took a lymph node from a case of a recurrent squamous epithelioma of the penis, ground it in a mortar and injected two cubic centimeters of it into the peritoneal cavity of a rat. The animal became emaciated and died fifteen months later. At the autopsy several nodules were found in the omentum, a large nodule in the liver and one in the spleen. The nodules were regarded as squamous epitheliomata, the cells being smaller than those of the primary tumor.

Dagonet and Mauclaire[23] took two cubic centimeters of a mixture of rectal carcinoma and salt solution and injected it into the peritoneal cavity of a rat. The animal became emaciated and was killed in six weeks. In the peritoneum were found several small ulcerating nodules. A second rat was similarly inoculated from the first. At the end of two months the abdominal cavity contained a large lobulated growth constituting about one third of the animal's weight. The microscopic structure was identical with that of the tumor in the first rat, but they both differed from the original columnar cell carcinoma. The authors designated the growth a *sarco-carcinoma.*

Lewin in discussing the above cases believes that in all those instances where the peritoneum was concerned the tumor-growths were nothing more than the result of an inflammatory reaction, namely, granulation masses. Von Hansemann[24] states that the transplantation of tumors from man to animal has never been successful. The reported positive results are easily explained by the facility with which the peritoneum in reacting to chronic irritation forms inflammatory tumors. The tumors which Jürgens obtained were nothing else than granulomata, and Dagonet's sacro-carcinoma was a pure inflammatory growth. In the other cases there may have been an accidentally associated tumor, as in Gaylord's. The tumor in the liver may not have been positively carcinomatous, and even supposing it to be it does not prove that it was due to the inoculation, as it represented the one positive result in many attempts. The carcinoma in the lung of Gaylord's guinea-pig was, according to von Hansemann, doubtless a benign adenoma such as have often been described as arising spontaneously.

Lewin[25] reports the following experiments on the transplantation of human cancer tissue into dogs. He took portions of a very rapidly growing ovarian

[21] Gaylord, cited by v. Hansemann, *Berl. klin. Woch.*, 1905, xlii, 313, 361.
[22] Dagonet, *Compt. rendu de la Societé biologie*, 1903, lv, 966.
[23] Dagonet and Mauclaire, *Arch. de méd. exper.*, 1904, xvi, 552.
[24] v. Hansemann, *Berl. klin. Woch.*, 1905, xlii, 313, 361.
[25] Lewin, *loc. cit.*

cancer and introduced them into the abdominal cavity of a dog. Three weeks later the animal was killed with chloroform and an autopsy made. There was found in the scar of a previous operation a tumor about the size of a joint of the little finger. This was quite firm and projected into the abdominal cavity. The peritoneum, especially the omentum and the mesentery, was covered with many small nodules varying in size from a pin point to a pin head, greyish-white in color and fairly firm. Some were also found on the gall bladder and the diaphragm. No neighboring zone of redness existed, the coils of intestine were not adherent, the peritoneum was smooth and shining and there was no fluid present in the cavity. The retro-peritoneal nodes were swollen, and although quite soft showed no caseation. One small but enlarged lymph node occurred behind the manubrium.

The microscopic examination showed the following: The peritoneal nodules are composed of masses of round cells between which lie a few with lobulated nuclei and spindle cells. Besides these are seen large, pale, endothelial nuclei. Vessels are present, no necrosis, no giant cells. The retro-peritoneal lymph node had the appearance of an inflammatory swelling. The scar tumor showed crossing strands of connective tissue with round and large spindle cells and lobulated leucocytes. In places the large cells were particularly numerous. Neither necrosis nor giant cells occurred.

Two dogs were inoculated with portions from the first growth. In the third generation four dogs were employed, two in the fourth and two in the fifth. In all instances further growths were obtained. In no case however was there a resemblance to the primary tumor and the author believes that in none was he dealing with a carcinoma. The structure was not typically sarcomatous although in many places it might be considered as such. In view of the work done by Ehrlich and Apolant,[26] in which sarcomatous tumors developed in mice as a result of the transplantation of cancer tissue, it would not be very surprising if a sarcomatous condition had followed in the course of the implantation of carcinomatous structures.

The possibility of the above tumors being infectious in character was considered and many examinations were made to determine that point. No organisms could be found by staining or by the inoculation of many forms of culture media. Portions of the growth were ground in a mortar and passed through a filter coarse enough to allow the passage of bacteria. Two dogs were injected with this material with negative results, while two other dogs were successfully inoculated with untreated pieces from the same growth.

A. Sticker[27] in numerous experiments, using goats, dogs, cats, rabbits, guinea-pigs, rats, and mice and inoculating in every possible way, was unable in so much as a single instance to transmit human cancer to the lower animals. In the course of time the inoculated juices and pieces of tissue were completely absorbed. The most careful macroscopic and microscopic examination failed to reveal any tendency toward tumor formation.

Leo Loeb[28] in a series of experiments on the transplantation of tumors from one rat to another made many attempts to determine if pieces of a rat

[26] Ehrlich and Apolant, *Berl. klin. Woch.*, 1905, xlii, 871; 1906, xliii, 37.

[27] Sticker, *Zeit. für Krebsforsch.*, 1904, i, 413.

[28] Loeb, *Jour. of Med. Research*, 1901, vi, 28; 1902, viii, 44.

sarcoma could be transplanted into guinea-pigs. All the attempts were unsuccessful. Equally unsuccessful were transplantations into white mice and into hens.

M. Herzog[29] tried for several years to produce carcinoma or sarcoma in rabbits and guinea-pigs by injecting triturated and filtrated human tumor material, subcutaneously, intraperitoneally, and into the anterior chamber of the eye, but has never had any success.

Mayet[30] made numerous attempts to inoculate lower animals with a glycerine suspension of human carcinoma. He concedes that his experiments with dogs and rabbits were negative but in his experiments with fifty-three white rats he says that he certainly did four times produce "epithelial cancerous lesions" and in eight more cases probably brought about similar changes. M. Herzog[31] in discussing the above says that "One cannot read Mayet's descriptions critically without becoming convinced that he has not succeeded in producing a true carcinoma in a single instance."

In view of the previous results of the transplantation of tumors of man to the lower animals the following experiment is recorded. The results indicate that possibly under certain conditions, at present unknown, inoculation may be successfuly achieved.

On November 3, 1906, a small portion of a scirrhus carcinoma of the breast was implanted, subcutaneously, in the abdominal wall of an adult white rat, the exact age of which was unknown, but the animal was quite a large one, and presumably fully grown. At the same time a like piece was implanted similarly in a white rat about four weeks old.

The following are brief notes of the two rats.

Rat 1. Piece of tissue implanted November 3, 1906.

November 7. Animal well. Small raised area at the seat of the implantation.

November 13. Lump in abdominal wall seems slightly larger. From this date on there was no change seen in the size of the nodule. At all times it was freely movable.

April 1, 1907. The rat was found dead. For the past couple of months it had been sneezing and coughing, had lost somewhat in weight and its coat was rough and dry.

Rat 2. Tissue implanted November 3, 1906.

November 7. Animal well, small raised nodule at the seat of

[29] M. Herzog, *Jour. of Med. Research,* 1902, viii, 74.
[30] Mayet, *loc. cit.*
[31] M. Herzog, *loc. cit.*

the inoculation. No evidences of an inflammatory condition.

November 13. Nodule has been decreasing in size and can at present no longer be felt. At the present date, August, 1907, the rat is perfectly well.

An autopsy was made on rat No. 1 with the following findings: On the abdominal wall at the site of the implantation there is a small whitish nodule about 9.25 mm. in diameter. It is not ulcerated, is firmly adherent to both the skin and the abdominal muscles and does not extend to the peritoneum. No other nodules are present.

The right lung is small, firm, and contained numerous small yellowish areas of a thick, semi-caseous material. Left lung is enlarged and emphysematous. The liver contains a small parasitic cyst (tænia). Other organs are apparently normal.

The microscopical examination of the abdominal nodule is as follows:

The specimen consists of a small oval nodule about 0.5 cm. long and one-half as wide, the end being blunt. On its outer periphery it is covered by a considerable thickness of skin and subcutaneous tissue. This surface is slightly raised above the adjacent normal structures. At the ends of the oval are found a few muscle fibers, some of which seem to be degenerated and replaced by connective tissue. The deeper portion rests upon the sheath of one of the abdominal muscles.

Surrounding this nodule is a very distinct capsule of adult connective tissue, the fibers of which run parallel to the surface of the foreign mass. In this capsule are found numerous small blood vessels; they are particularly well marked along the subcutaneous periphery.

In and beneath this capsule there is found an almost continuous line of cells of two varieties. One of these contains a small round deeply staining nucleus about which is very little protoplasm; they are probably lymphocytes. The second form is a cell containing a long nucleus that varies considerably, both in thickness and in its staining capacity. Some of the nuclei are very thin and stain deeply. The larger nuclei are oval and more vesicular in character.

The long, narrow, and deep staining nuclei lie within and run parallel to the connective tissue fibers surrounding the nodule.

The fibers forming the capsule seem to consist of the pre-existing connective tissue compressed by the nodule. In a few places fibers appear to branch off from the capsule and extend a short distance into the nodule. Their identity is, however, soon lost.

Lying within the peripheral zone of the nodule and extending more or less in a right angle are many cells containing large oval or spindle-shaped vesicular nuclei. There are also found, similarly situated, numerous narrow deeply staining nuclei. In the majority of instances the nuclei are found in what appear to be interstices of the tissue.

At one place on the upper portion of the nodule there is a large area of infiltration by cells containing small round deeply staining nuclei. There are also many small newly formed capillaries in this region. Beyond the area of round cell infiltration are many oval, faintly staining, bubble-like nuclei. These have no definite arrangement, are scattered quite irregularly. Numerous collections of these larger vesicular nuclei formed of a dozen or more individuals heaped up together without any visible cell protoplasm or cell wall are also present.

The greater part of the nodule, excepting only a narrow peripheral zone, has undergone a marked degeneration. No nuclei are evident nor can any formed structures be distinguished. There are, however, certain distinct differences in the density of the tissue evident in some portions. Bands of faintly staining tissue are found extending in all directions. Between these are areas, varying greatly in shape and size, that are much less dense and which show no trace of a fibrillar structure. They appear to be filled with a distinctly granular material. In a few instances there can be seen among the granular debris small round bodies that are homogeneous and have taken a very faint bluish tinge.

On comparing this nodule with the original human specimen it is quite evident that the fibrillar bands represent the primary connective tissue, while the granular areas are the remains of the epithelial nests.

In these two cases we see that in the young rat there was within ten days a complete absorption of the implanted tissue with no further manifestations. In the older rat there was a decidedly

different course of events. The cancerous nodule was not absorbed but the bodily resistance of the rat appeared to be sufficient to prevent any increase in size or extension into other parts. The foreign tissue was walled off by a connective tissue capsule, no new blood vessels were formed and the pre-existing ones failed to obtain nourishment for the cellular structures. Consequently there was a degeneration of the epithelial elements. The connective tissue being more resistant underwent a slighter degeneration with little or no absorption.

It would, therefore, seem evident that while there was something in the younger rat that very quickly brought about the destruction of the foreign tissue, in the older rat the necessary conditions were lacking. It would therefore appear that the nodule remaining unabsorbed in this rat for five months, that a less degree of resistance of the rat, or an increased degree of virulence of the tumor might render a growth of the implanted tissue possible.

RIGOR MORTIS AND THE INFLUENCE OF CALCIUM AND MAGNESIUM SALTS UPON ITS DEVELOPMENT.*

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THE THEORIES AND SOME OF THE FACTS OF RIGOR MORTIS.

Historical.—Two features of the phenomenon of rigor mortis, the stiffness and the shortening of the skeletal muscles, bear a resemblance to two physiological processes: the clotting of the shed blood and the contraction of living muscles. Upon the basis of this resemblance two divergent theories were brought forward as far back as nearly a century ago. These theories are the ones which at the present day are still competing for supremacy.

The Contraction Theory.—Nysten(1) (1811) thought rigor to be "le dernier effort de la vie contre l'action des forces chimiques." Denuded of its vitalistic garb, the phrase expresses the view that rigor is due to a physiological contraction of the muscles. Although Brücke(2), one of the grave-diggers for the theory of vital force, rejoiced as far back as 1842, at the prospect that the theory of the "dernier effort de la vie" was then buried forever, the theory of contraction was soon resurrected, and even at the present day is occupying a well-supported position in the domain of physiology. Besides Schiff(3), Brown-Sequard(4) and some others, it was especially L. Hermann(5) and his pupils who brought together many facts in favor of the assumption that rigor mortis shows a certain form of muscular contraction.

Coagulation Theory.—As to the coagulation theory it was assumed originally by Orfila(6), Treviranus(7) and others that rigor was due directly to the clotting of the blood and lymph between the

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muscle fibers. This theory was favored also by Johannes Müller(8) for the reason that the contraction of the clot and the separation of serum from it would also explain the final release of the muscles from rigor. This theory, however, had to be given up as soon as it was recognized that rigor also occurs in completely exsanguinated animals. But Brücke offered the same theory in a modified form. Soluble fibrin, said he, enters during life into the muscle fibers and it is the clotting of this intrafibrillar fibrin which is the underlying cause of postmortem rigor. Virchow(9) called attention to the fact that the plasma obtainable from muscle differs chemically from the contents of the blood, and Brücke himself and others failed in the attempt to obtain a plasma from the muscles before they entered into a state of rigor; the chemical theory had therefore for a long period very little support from experimental facts. But in 1859 Kühne(10), who carried out all the necessary manipulations at low temperatures, finally succeeded in obtaining a liquid muscle plasma which gradually clotted spontaneously. The coagulum, which differs from the fibrin of the blood, Kühne termed myosin.

The theory that rigor is due to a sort of coagulation and resembles clotting, or in short the chemical theory of rigor, had now a solid fact for its foundation; the muscles, like the blood, while in a living state, contain a soluble proteid which clots spontaneously some time after the removal from its natural connections. That the rigor is due to the clotting of that proteid within the muscles is, of course, not a fact but only a hypothesis, but a hypothesis which is greatly supported by the following facts: that the spontaneous clotting of the myosin occurs about the same time after death as the muscles, when kept under similar conditions as the plasma, enter into rigor; that further, muscles, from which myosin is pressed out, no longer enter into rigor, and that finally the myosin obtainable from muscles already in rigor is considerably less in quantity than that obtainable from normal muscles. All these facts were brought out by Kühne himself.

Some Facts Favoring the Contraction Theory.—In the half century which passed since the establishment of the chemical theory by Kühne no new facts came to light which essentially strengthened its position, although the knowledge of the chemical nature of

myosin was considerably advanced by many investigators, especially by the extensive studies of Halliburton(11) and von Fürth(12). On the contrary, new questions arose to which the chemical theory is as yet incapable of giving a satisfactory answer. For instance, it is now well established that the clotting of blood plasma occurs under the influence of a well-defined ferment; but no ferment could be discovered, in spite of an eager search for it, which was instrumental in the clotting of muscle plasma(13). Furthermore, is it now shown that the release from rigor is not due to the setting in of putrefaction, as was believed formerly (14 and 15); the release from rigor is as spontaneous an act and as vital a phenomenon, so to say, as its onset. But the once clotted myosin fibrin and myogen fibrin, as the clotted proteid bodies from the muscle plasma are now termed by von Fürth, do not show any reversibility, do not become soluble again. Neither did the vigilant search lead to a detection of a ferment which might be concerned in the release of the muscles from rigor(13). The release reminds one rather of the final relaxation of a muscular contracture. Another fact, difficult to understand on the basis of the chemical theory, is the disappearance of rigor, at least at a certain stage, by bending or kneading the muscles. It was further established that the time of onset, the intensity and duration of rigor differ considerably between white and red muscles (14 and 16). It is known that these muscles differ in the character of their contractility. But it is not known that there is any difference in the fibrin bodies obtained from these muscles. Moreover, the onset and course of rigor depend a great deal upon the antemortem functional state of the muscles; for instance, the antemortem destruction of parts of the central nervous system(17), or cutting of the motor nerves(18), or fatigue of the muscles(19), or their paralysis by curare(20) retard strikingly the onset of rigor; while, after stimulation of motor nerves(21), or after poisoning by strychnin(22), or in deaths due to toxic tetanus(23) the development of rigor is greatly accelerated; all these are facts which indicate an intimate connection of the postmortem rigor with the antemortem muscular contractions.

Various Forms of Rigor.—The numerous studies devoted to the subject brought to light many other valuable and instructive facts,

but it would seem that this knowledge rather increased than diminished the difficulty of interpreting postmortem rigor. In the first place we have to mention other forms of rigor which are to be distinguished from rigor mortis.

1. An extremity of a living animal gets into a state of rigor if its circulation is arrested (Stenon's experiment). This form of rigor, if not too far advanced, is reversible by restoring the circulation.

2. Rigor can further be produced by submersing a muscle in water, or, still better, by injecting water into the arteries of muscles—water rigor (24).

3. By exposing muscles to higher temperatures, they pass rapidly into a state of rigor—heat rigor (25). Quite a large literature grew up on the subject of heat vigor, and there is quite a divergence of opinion as to the nature of the rigor as well as to the degrees of the temperature which cause it in cold-blooded as well as in warm-blooded animals. However, it seems quite certain that rigor occurs at a temperature which is far below the one sufficient to coagulate all the albuminous bodies. Whether there is any relation between these temperatures and the coagulation temperatures of the myosin bodies has not been satisfactorily established. It seems to us that in the literature on heat rigor the conceptions of coagulation and clotting have not always been sharply kept apart,—a confusion against which Kühne had already warned.

4. Rigor can be produced by freezing temperatures—cold rigor (Folin).[1]

[1] Folin (*American Journal of Physiology*, 1903, ix, 374) states that Brücke (*Müller's Archiv für Anatomie und Physiologie*, 1842, 176) described rigor produced by cold. But Brücke claims, against Sommer, that rigor persists after thawing and he says nowhere that freezing brings on rigor; on the contrary, he claims that rigor sets in before the freezing takes place. The single experiment mentioned by Brücke in which the amputated thigh of a frog, after being submersed in distilled water and kept in a freezing mixture, was found to be in a state of rigor after thawing, is surely insufficient evidence for a claim that rigor is produced by cold; the rigor could have been due to the distilled water—water rigor.

Folin says further that "Brücke's experiments with cold as a means of producing rigor received, curiously enough, no attention from subsequent investigators and as far as I have been able to learn the discovery has been forgotten." Folin overlooked a paper by L. Hermann (*Pflüger's Archiv*, 1871, iv, 188) en-

5. By injections of such substances as chloroform, ether, caffein-benzoate, etc.(26), directly into the muscles or intravenously, the muscles pass immediately into a state of strong rigor—chemical rigor.

Definition of Rigor.—Another puzzling question is: What constitutes rigor? Many deviations from the normal are met in rigor mortis: the muscles are stiff and of denser consistency than normal; their elasticity differs from that of normal muscles; they show an acid reaction, their irritability is lost, etc. Are all these changes essential parts of rigor and of every kind of rigor? Some believe that shortening of the muscles does not constitute an integral part of rigor. Many authors claim that the irritability of the muscle might persist even after the release from rigor(27). According to others the acid reaction is not indispensable for the development of rigor(28). Folin finally, as we have seen above, considers hardness and stiffness alone as the essential criteria of rigor.

We are apparently not yet ready for a final answer. A century of work has brought no decision between the contending theories. It has brought to light a great many single facts and that is what

titled: Die Erstarrung in Folge starker Kältegrade. This is the more regrettable as the knowledge of this paper might have been not without some influence upon Folin's method of investigation as well as upon his conclusions. As Folin did not return to this work again, as he intended to do, it might not be amiss to discuss here some of the essential points. Folin says that frog muscles which were cooled to — 15° C. were found on thawing to have gone into rigor. The rigid muscles are perfectly translucent—not opaque—and apparently are not shortened. The first important conclusion drawn by Folin from these observations is that the only fundamental characteristic of rigor is the stiffness and hardness of the muscles and that opacity and shortening of the muscles, evolution of carbon dioxide, etc., are unessential incidents of the main phenomenon. On the implicit assumption that the muscles, while they are still frozen, are already in a state of rigor, extracts were prepared from them—before thawing—and compared with extracts made from normal muscles before rigor. It was found that the spontaneous coagulation as well as the heat coagulation was the same in both extracts, and that there was no difference with regard to the degrees of acidity as well as in the amounts of the total nitrogen. Since one of the extracts was made from muscles in rigor, Folin draws the conclusion that coagulation cannot be the cause of rigor mortis. It must be remembered that this conclusion is based upon the supposition that the muscles are in a state of rigor while they are still frozen. Now Hermann says that a thoroughly frozen muscle "verfällt nach dem Aufthauen einer beschleunigten Erstarrung," that is the rigor occurs *after* thawing; the freezing has only the effect of hastening

we still need and can accomplish at present: the uncovering of some more facts—the collection of more bricks for the erection of a structure at some future time.

THE RELATIONS OF CALCIUM AND MAGNESIUM TO RIGOR

Introductory.—The aim of our paper is the contribution of a few facts regarding the relations of calcium and magnesium to the phenomenon of rigor mortis. What are these relations? On *a priori* grounds our expectations may differ with our conceptions of the nature of rigor. From the point of view of the chemical theory we might expect that the mentioned alkali earths are capable of exerting a definite influence upon the development of rigor, but that the character of the influence might be different for both substances. It is now well established that calcium salts hasten the clotting of blood. We do not know of a similar effect from magnesium salts; on the contrary, concentrated solutions of magnesium sulphate are employed to prevent or retard the clotting of blood. Do both substances behave in a similar manner towards the clotting of the muscle plasma and also towards the onset of rigor? That is, does

the onset of the rigor. Hermann says further that the thawed-out and not yet rigid muscle has a different appearance from the normal; it is translucent, and the (final) rigor of such frozen muscles differs from that of normal muscles by the *extreme degree of shortening and thickening* and by the exudation of a strongly acid serum. According to Hermann the rapid onset of the rigor does not depend upon thawing, or upon the degree and duration of the freezing, but upon the rapidity of the development of the latter. The essential points which are of interest to us here are, that according to Hermann the muscles, while they are still frozen, are not yet in a state of rigor, but that the freezing hastens greatly the onset of rigor, that the not-shortened translucent muscle is not yet in a state of rigor and that the real rigor which follows that stage is rather marked by an extreme shortening.

We offer of course no opinion as to which of the observations and views are the correct ones. But it seems to us that to uphold the conclusions of Folin the experiments ought to be repeated and the extracts ought to be made not from frozen muscles but after they were thawed out for some time, that is while they are in a state of undisputed rigor.

As to the question whether there is any difference between extracts made from muscles in rigor and from normal muscles, we may refer here to the recent investigations of Saxl (*Hofmeister's Beiträge zur Chemischen Physiologie,* 1906, ix, 1). He found that the proportion of "muscle plasma" obtainable from muscles in a state of postmortem rigor to that obtainable from fresh muscles is about 1:3.

calcium hasten the clotting of the plasma and also hasten the onset of the rigor, and does magnesium retard both? On the other hand, from the point of view of the contraction theory there are some reasons to expect that both ions might rather retard the development of rigor. It is well established, as we have mentioned above, that substances which increase muscular activity hasten the onset of rigor, while those which impair the contractions, curare, for instance, retard its onset. Now Loeb(29) has maintained that calcium exerts an inhibitory influence upon muscular contraction. A similar inhibitory influence Loeb ascribes also to the entire group of alkali earths except barium. We ourselves(30) have published several communications on the inhibitory effect of magnesium salts. But if calcium as well as magnesium is capable of inhibiting muscular contractions, rigor being in the nature of a contracture, we may expect that the effect of either ion would be in the direction of retardation of the development of rigor.

There are in the literature a few scattered statements bearing upon our question, more upon the effects of calcium than upon those of magnesium. Cavazani(31) reported that muscles poisoned by potassium oxalate do not pass into a state of rigor. As the oxalates precipitate calcium salts, Cavazani concludes from his observation that rigor is due to the presence of calcium salts in the muscles. However, neither Howell(32) nor Locke(33) were able to confirm the statement of Cavazani: muscles perfused with or kept in oxalate solutions passed into a state of rigor at least as early as the controls. From these experiments it could appear that calcium is of no importance in the development of rigor; but it must be remembered that potassium oxalate probably does not remove all the calcium from the muscles. Furthermore, Howell(34) himself found for heart muscle (strips of terrapin's heart) that a surplus of calcium chloride leads to rigor. Von Fürth(35), in studying the effect of many substances upon the clotting of muscle plasma and upon the development of rigor, found that the addition of calcium chloride, calcium nitrate or magnesium nitrate accelerated the clotting of a myogen solution, while the addition of magnesium sulphate did not exert such an influence. However, perfusion of the posterior extremities of frogs with ten per cent. solutions of calcium chloride or calcium nitrate did not hasten the onset of rigor.

A. Moore(36), working in J. Loeb's laboratory, tested the effects of various crystalloids upon rigor mortis by bathing the gastrocnemius muscles of frogs in solutions of these substances. For calcium chloride is was found that "a strong solution has at first a relaxing effect, but after a period varying from half an hour to one hour and a quarter, contraction begins, the lever reaching its maximum height in from three quarters to four hours." "In weaker solutions, $= n/2$, $= n/4$, contraction is slower in beginning and slower in setting, but as the strength of the solution is further decreased, rigor takes place more quickly." For magnesium chloride it is stated "that the effect is relaxing. It not only does not cause rigor, but actually prevents it." "Contrary to the above statement, however, rigor seems to be produced in the strength $= n/2$." For magnesium sulphate it is said that "no immediate effect was produced on the muscles immersed in the solution. A gradual rise began soon, however, indicating the approach of rigor, and the muscle assumed the usual rigor appearance. Rigor was noted in solutions stronger than $= n/4$."

In the extensive work of Overton(37) on the effect of different crystalloid solutions upon muscle and nerve the word rigor (Starre) does not occur, although frog muscles were kept in various solutions, sometimes for several days. We find, however, in the protocols (vol. 105, p. 220), that a frog muscle, kept in a solution of calcium chloride which is isosmotic with 0.7 per cent. solution of sodium chloride, about nine hours after removal from the body is "completely unirritable, shortened from thirty-eight to fifteen millimeters, opaque and very little plastic"; that can only mean that the muscle is in complete rigor about nine hours after death, which is for a frog muscle, kept at a temperature of 9° C., a very early onset.

Finally, Cushing(38) has found that the injection of Ringer's solution into the belly of a gastrocnemius muscle of a living frog may cause rigor which he ascribes to the presence of the calcium ion in the solution and compares this rigor with the calcium rigor of a strip of heart muscle described by Howell.

According to Moore, then, magnesium chloride as well as calcium chloride has more or less a relaxing effect upon the rigor of frog

muscles. These observations would seem to support, as argued above, the contraction theory of rigor. Moore, however, leans rather towards the chemical theory.

EXPERIMENTAL STUDIES BY THE WRITERS.

Method.—We made no observations upon the effect of bathing single muscles in the salt solutions. Our studies were made by injecting the solutions into living animals. The observations were made at first incidental to other experiments with magnesium and calcium salts. Injections were made into various species: dogs, cats, rabbits, guinea-pigs, rats and frogs. In the studies devoted directly to the subject of rigor mortis the most extensive observations were made upon rabbits. We experimented, however, also upon cats and frogs. In mammals the injections were made, at least in the main study, nearly exclusively intravascularly. In the frogs injections were also made into the lymph sacs. The intravascular injections in mammals were carried out by two methods: the intravenous and the intra-arterial. We shall report first the results we have obtained by intravenous injections.

Intravenous Injections of Calcium and Magnesium Salts.—The method of intravenous injection needs no special description. The solutions were permitted to run slowly into the external jugular vein from a burette arranged on the principle of a Marriotte's flask. Before beginning the infusion the animals were provided with a tracheal cannula and a cannula in the left external jugular vein. This, as well as any other operative procedure which may be mentioned later, was carried out under ether anesthesia. Usually two animals were studied at the same time, one for a magnesium and one for a calcium salt, the animals serving as controls for one another. In the magnesium animals artificial respiration had to be started pretty soon after beginning the infusion. But with artificial respiration and slow infusion a fairly good quantity can be introduced before the heart stops beating, the quantity, of course, depending upon the concentration of the solution.

In each double experiment the same molecular concentration of both salts was employed, and as nearly as possible the same quantity was infused into each animal. In the calcium animals the

respiration was rarely impaired during the entire time of the infusion, but they frequently received artificial respiration, nevertheless, in order to have similar conditions in both. The magnesium animals usually died from the infusion; the calcium animals mostly had to be killed at the end of the infusion, the animals being killed by opening the thorax. The solutions were employed as indicated above according to molecular concentrations, the weakest solution used being $= m/8$ and the strongest $= m/1$. The time elapsing between the death of the animal and the onset of rigor in the various parts of the body was taken as the main unit of comparison between the effects of the salts. The time of death is well marked, of course, by the final standstill of the heart. As to the appearance of rigor a distinction must be made between the fully developed state and its beginning, and between the rigor of the whole animal and that of the various parts. There is not the slightest doubt about the presence of rigor when it is in an advanced state, but its exact beginning is not always above dispute. Other difficulties with which one is confronted in this kind of observation are caused by variations in the time of onset of rigor in the individual animals, even under apparently exactly the same conditions, and by some variations in the order with which the rigor appears in the several parts of the body. However, the results obtained in our experiments were of such a palpable degree as to be in every instance above any doubt.

We shall now report our results, illustrating each series by abbreviated protocols of a few of the experiments.

ABBREVIATED PROTOCOLS OF SOME EXPERIMENTS.

Series I. Intravenous Infusions of the Salts in Molecular Solutions.

EXPERIMENT 1.—Magnesium chloride. Female rabbit, 1060 grams. Ether; tracheotomy; cannula in external jugular vein, connected with burette which contained magnesium chloride in $M/1$ solution.

11.55 a. m. Infusion begun. When 1.5 c.c. ran in, spontaneous respiration disappeared; started artificial respiration.

11.59 p. m. 4.5 c.c. ran in; animal dead. Thorax opened; left ventricle moderately contracted, right dilated and soft.

2.10 p. m. Left ventricle relaxed again. No stiffness in jaws, neck or legs.

3.55 p. m. Jaws locked; perhaps slight stiffness in neck; extremities still soft.

4.40 p. m. Neck stiffer; some stiffness of front and hind legs.

6.00 p. m. Neck stiff; rigidity of front and hind legs has progressed, but is far from being complete. (No further note on heart.)

The next day at 10.30 a. m. the animal is quite rigid.

In this experiment with magnesium chloride the jaws became rigid about four hours after death and at that time the rigidity of the neck just began. The rigidity of the legs began about four hours and forty minutes after death. It was not observed at what time the rigor attained its maximum, it was far from it six hours after death; it was found, however, to be complete next morning.

EXPERIMENT 2.—Magnesium sulphate. Female rabbit, 1140 grams. Same preparation as in previous experiment; burette was filled with magnesium sulphate in *M*/1 solution.

12.21 p. m. Infusion begun; artificial respiration started soon after.
12.30 p. m. Heart stopped, 6.5 c.c. infused.
2.10 p. m. No stiffness of any part of body, heart soft, moderately dilated.
3.55 p. m. Jaws locked; neck soft.
4.40 p. m. Neck soft; front legs soft; hind legs slightly stiff at hips.
6.00 p. m. Neck getting stiff; stiffness in all four legs, increased in hind legs.
Next day at 10.30 a. m. legs and neck are rigid.

In this rabbit (magnesium sulphate) the jaws were locked after about three hours and a half, while rigidity of the neck did not begin until five and a half hours after death. Also the rigidity of the legs was more retarded in this animal than in the magnesium chloride rabbit.

EXPERIMENT 3.—Calcium chloride. Female rabbit, 1200 grams. Burette filled with calcium chloride in *M*/1 solution.

12.48 p. m. Infusion begun.
12.54 p. m. Respiration good, but artificial respiration started.
12.57 p. m. Heart stopped. Infusion stopped, 5 c.c. infused.
1.10 p. m. Heart contracted, left ventricle more than right.
2.10 p. m. Neck quite stiff; rigor in hind legs, good stiffness on extension at knee.
2.45 p. m. Full rigor in hind legs.
3.55 p. m. Rigor quite marked in front legs.
4.40 p. m. Complete rigor in entire body (no note on jaws).
Next day at 10.30 a. m. the body is less stiff and the neck is soft.

In this animal (calcium rabbit) the neck was quite rigid one hour and fifteen minutes after death, and at that time the rigidity of the hind legs also was fairly established. In the front legs a marked rigidity was present about three hours after death. About four hours after death the entire rigor was complete. But next morning the release from rigor had already begun.

We shall add one more protocol of a calcium experiment, carried out with a *M*/1 solution.

EXPERIMENT 4.—Calcium chloride. Female rabbit, 2000 grams. Same preparation; right sciatic nerve cut at exit from pelvis; burette filled with calcium chloride in *M*/1 solution.

12.20 p. m. Infusion begun, given very slowly; artificial respiration started.

12.39 p. m. Convulsions appeared.

12.43 p. m. Infusion stopped, 8 c.c. infused. Artificial respiration stopped; no convulsions; heart continues beating freely for one minute. Soon after heart dilated and flabby.

1.00 p. m. Left ventricle contracted, right soft.

1.30 p. m. Neck quite rigid; jaws can just be opened with force; front legs show slight stiffness on flexion; hind legs some resistance on flexion; definite rigor on extension at hips; right leg stiffer than left.

2.00 p. m. Jaws locked; neck more stiff; marked stiffness of front legs on flexion; rigidity of hind legs increased.

5.15 p. m. Stiff as a board everywhere; right hind leg fully extended; left hind leg more flexed.

Next day at 10.00 a. m. neck is soft, jaws can be opened and the entire animal shows good relaxation—more so than another rabbit which received on the previous day only 4 c.c. of calcium chloride in *M*/1 solution.

In this (calcium) animal the rigor was everywhere fairly established about forty-five minutes after death. The maximum rigor of the entire animal was attained in four and a half hours. Next morning, however, the release from rigor had already begun and was considerably advanced.

In this series with intravenous infusion of molecular solutions the onset of rigor in the animals receiving magnesium chloride and magnesium sulphate was delayed by a few hours in comparison with the onset of rigor in the calcium animals. In the latter the onset as well as the complete development of the rigor appeared quite early and the earlier the larger the infused quantity was. The release from rigor appeared earlier in the calcium than in the magnesium animals.

Series II. Intravenous Infusions of M/2 Solutions.

EXPERIMENT 4.—Magnesium chloride. Female rabbit, 1700 grams. Preparation same as in previous experiments; burette filled with magnesium chloride in *M*/2 solution.

11.09 a. m. Infusion begun.

11.10 a. m. Respiration shallow; artificial respiration started.

11.20 a. m. 19 c.c. ran in; occasional heart beats; artificial respiration stopped; animal dead (thorax not opened).

12.42 p. m. No stiffness anywhere.

12.50 p. m. No definite stiffness.

5.00 p. m. Moderate, but definite stiffness of hind and front legs, more in hind legs.

Next day, 11.00 a. m., animal stiff; no retraction of head, and legs not extended; right ventricle of heart dilated and soft, left ventricle moderately contracted. No relaxation of body set in during the day.

Five and a half hours passed before any sign of rigor appeared. The complete rigor observed next day was of a flexion type. There was no relaxation next day.

EXPERIMENT 6.—Calcium chloride. Female rabbit, 1800 grams. Burette filled with calcium chloride in *M/2* solution.

12.01 p. m. Infusion begun; no artificial respiration.

12.14 p. m. 19 c.c. ran in; infusion stopped. Irregular respiration; feeble heart beats; a few convulsive struggles.

12.16 p. m. No heart beats felt; dead, thorax not opened.

12.42 p. m. Definite stiffness of hind legs; front legs soft.

1.15 p. m. Definite stiffness of front legs also.

2.15 p. m. Entire animal rigid.

5.00 p. m. Animal lying with head retracted, all four legs strongly extended and with marked lordosis of back.

Next day at 11.00 a. m. the animal is in the same position, but is not so stiff; both ventricles are strongly contracted.

1.30 p. m. No longer as rigid as on previous day; especially front legs soft again.

Twenty-six minutes after death of this calcium animal definite rigor was present; the animal showed later a strong rigor in extreme extension, but the release from rigor had already begun early on the following day.

Experiments on cats with *M/2* solutions brought out the same differences between calcium and magnesium chloride observed in rabbits: early onset, and development of rigor of an extension type with a comparatively early release, in the calcium animal, and late onset, and development of rigor of a flexion type, with a late release in the magnesium animal. The left ventricles showed the same differences as the skeletal muscles.

Series III. Intravenous Infusions of M/4 Solutions.

EXPERIMENT 7.—Magnesium chloride. Male rabbit, 1180 grams. Same preparation; burette filled with magnesium chloride in *M/4* solution.

11.34 a. m. Infusion begun, running slowly.

11.38 a. m. 5 c.c. infused; respiratory struggles; artificial respiration started.

11.50 a. m. Stopped infuson, 26 c.c. infused; heart just perceptible; stopped artificial respiration.

11.52 a. m. Thorax opened; heart not beating.

1.00 p. m. All parts of body flaccid, heart soft.

1.55 p. m. Same.

2.55 p. m. Jaws cannot be opened; neck and extremities soft.
3.50 p. m. Same.
5.30 p. m. Rigor beginning in hind and front legs, neck still soft.
Next day all parts in rigor; unchanged the entire day.

In this animal rigor began in the jaws and muscles after three hours and in the legs after five and a half hours, while the neck was still soft. There was no relaxation next day.

EXPERIMENT 8.—Calcium chloride. Female rabbit, 1690 grams. Same preparation; burette filled with calcium chloride in *M*/4 solution.

12.09 p.m. Infusion begun.
12.23 p. m. 15 c.c. infused. Heart slightly irregular; started artificial respiration.
12.30 p. m. Stopped infusion; 26 c.c. infused; stopped artificial respiration; heart good, but no respiration—apnœa.
12.32 p. m. Breathes; opened thorax; death under signs of asphyxia.
1.25 p. m. Both ventricles contracted; body flaccid.
1.55 p. m. Jaws and neck rigid.
2.55 p. m. Marked rigidity of entire body.
3.50 p. m. Extreme rigor.
Next day at 12 m., neck and front legs are soft.

In this (calcium chloride) animal the rigor began in the jaws and the neck after one hour and twenty minutes and in the rest of the body after two hours and twenty minutes. The rigor attained its maximum, however, in three and a half hours. The release began early the following day.

EXPERIMENT 9.—Magnesium nitrate. Male rabbit, 2450 grams. Same preparation; burette filled with magnesium nitrate in *M*/4 solution.

1.08 p. m. Infusion begun.
1.18 p. m. 13 c.c. infused; respiration shallow; artificial respiration started.
1.31 p. m. 32 c.c. infused; heart quite weak; stopped infusion for three minutes.
2.00 p. m. Stopped infusion, 51 c.c. infused; stopped artificial respiration; no reaction whatsoever.
2.05 p. m. Thorax opened; heart beats; a good deal of bleeding.
2.15 p. m. Heart stopped beating; everything soft; blood clots poorly.
3.50 p.m. Jaws slightly stiff, everything else flaccid.
5.00 p. m. Jaws locked; every other part of body quite soft. Both ventricles soft; right ventricle contracts on touch.
6.00 p. m. No change.
8.30 p. m. Slight rigor in legs, not pronounced; very slight stiffness in neck, if any; left ventricle rather strongly contracted.
Next day, rabbit is very stiff and rigor is of a flexion type.

In this (magnesium nitrate) animal there was even after six hours practically no rigor in extremities and neck, the jaws however

already began to show stiffness one hour and thirty minutes after death.

EXPERIMENT 10.—Calcium nitrate. Female rabbit (weight ?). Usual preparation; burette filled with calcium nitrate in $M/4$ solution.

10.50 a.m. Infusion begun.

11.10 a. m. 15 c.c. infused so far; respiration poor; inspiratory tonus; started artificial respiration.

11.25 a. m. Stopped infusion, 25 c.c. ran in. Thorax opened, no heart beats.

11.08 a. m. Jaws locked; neck slightly stiff; moderate but definite stiffness in front legs.

12.10 p. m. Some stiffness in hind legs; left ventricle strongly contracted.

12.50 p. m. Front legs extended; stand out like sticks, hind legs very stiff, but still flexed. (Not observed any further.)

This animal received of calcium nitrate $M/4$ about one half of the quantity which the animal in the foregoing experiment received of magnesium nitrate. The contrast between the two animals was very striking. While in the magnesium animal there was no rigor even six hours after death, there was in the calcium animal a definite rigor already thirty minutes after death.

EXPERIMENT 11.—Calcium acetate. Male rabbit, 1850 grams. Same preparation; burette filled with calcium acetate in $M/4$ solution.

2.15 p. m. Infusion begun.

2.32 p.m. 10 c.c. infused; lid reflex active; respiration good, but heart cannot be felt; convulsions set in. Artificial respiration started and thorax opened. Heart beats extremely rapidly, left ventricle almost in complete tetanus; very slight diastolic relaxations. (Artificial respiration, though strong, produces no apnœa.) Six cubic centimeters more given.

2.40 p. m. Heart stopped; animal dead. Heart soon became soft.

2.50 p. m. Heart beginning to show rigor, progressing from base to apex; was completed in three minutes; right ventricle remains soft, auricles pale.

3.05 p. m. Neck, jaws and four legs in good rigor. The rapid progress could be directly perceived; the legs were seen moving, shortening, sometimes in short starts.

4.30 p. m. No change; complete rigor.

Next day at 3 p. m., the neck was soft, also jaws and legs a good deal softer.

The development of the rigor in this (calcium acetate) animal was a striking spectacle. Twenty-five minutes after death the rigor was complete, and the progress was a matter of ocular perception.

In this series the magnesium nitrate had the most delaying, and calcium acetate a most accelerating effect upon the onset and development of rigor.

Series IV. Intravenous Infusion of M/8 Solutions.

EXPERIMENT 12.—Magnesium chloride. Female rabbit, 1445 grams. Same preparation; right sciatic nerve cut; burette filled with magnesium chloride in *M*/8 solution.

11.00 a. m. Infusion begun.

11.01 a. m. Started artificial respiration.

11.05 a. m. Infusions stopped; 30 c.c. infused.

11.07 a. m. Thorax opened; heart beats occasionally.

12.50 p. m. Left ventricle slightly contracted; everything soft otherwise.

3.20 p. m. Both hind legs moderately stiff, right more than left; front legs show beginning of rigor.

4.00 p. m. Both hind legs stiff, now left more than right; front legs stiffer than before; left ventricle well contracted, right still dilated and soft.

5.30 p. m. All legs very stiff; no difference between hind legs.

Next day at 2.30 p. m., front legs are soft, hind legs stiff.

In this animal with a solution of magnesium chloride nearly isosmotic with a "physiological" sodium chloride solution, the rigor began earlier than with the more concentrated solutions—about three hours after death, attaining its maximum in about six hours and showing already in the afternoon of the next day a distinct beginning of release from rigor.

EXPERIMENT 13.—Calcium chloride. Female rabbit, 1370 grams. Same preparation; right sciatic cut; burette filled with calcium chloride in *M*/8 solution.

11.27 a. m. Infusion begun.

11.37 a. m. Stopped infusion; 30 c.c. infused; convulsions; thorax opened; heart still beating.

12.40 p. m. Neck stiff, front legs, especially right, stiff; stiffness of hind legs beginning; no difference between right and left.

12.50 p. m. Right and left ventricles strongly contracted.

1.20 p. m. Left hind leg definitely stiffer than right.

5.30 p. m. Legs in full rigor, no difference between hind legs. Next day in afternoon all legs more or less soft again.

In this (calcium) animal the onset of rigor occurred also pretty early, about one hour after death, but the maximum was not attained until about six hours after death. The onset of rigor in the right ventricle occurred unusually early, after about one hour and ten minutes.

Transfusion with Sodium Chloride.—We append here an abbreviated protocol of one of the experiments made with transfusion of sodium chloride in molecular solution. It may serve as a control experiment to the foregoing observations.

EXPERIMENT 14.—Sodium chloride. Male rabbit, 2039 grams. Same preparation; burette filled with sodium chloride in *M*/1 solution.

12.43 p. m. Infusion begun.

12.55 p. m. Stopped infusion; 7.5 c.c. infused; animal showed not the slightest reaction; heart, respiration, lid reflex, etc., unchanged.

12.57 p. m. Killed by clamping the trachea. When the convulsions due to asphyxia were over, the thorax was opened; heart beating feebly.

1.10 p. m. Heart beating slightly.

1.45 p. m. Left ventricle well contracted; everything else flaccid.

2.05 p. m. Jaws show good stiffness; some stiffness on extension at elbow and thigh.

3.20 p. m. Neck rigid; front legs stiff on extension and flexion; hind legs very rigid at hip on extension; beginning rigor in other parts.

3.50 p. m. Practically stiff all over.

Next day, 3.30 p. m., neck soft, other parts also less rigid.

In these cases the rigor set in later than in the calcium and earlier than in the magnesium animals.

The sodium chloride animals were very little affected by the transfusion, and at the end of it had to be killed. The killing was done either by clamping the trachea or by free opening of the thorax, and the asphyxia brought on the usual antemortem convulsions. Now since any kind of convulsion hastens the onset of rigor, and since magnesium animals die without convulsions, the question arose, whether it was not these convulsions which made the rigor appear earlier in the sodium chloride animals than in the magnesium rabbits. Furthermore the very same question had to be met with regard to the accelerating effect of calcium, since the calcium salts did not paralyze the animal and frequently indeed convulsions appeared either in the course of the transfusion or shortly before the death of the calcium animals. In other words, we were confronted with the question whether the difference in the time of onset of the rigor between the calcium and magnesium animals was not due simply to the fact that magnesium paralyzes the animal and calcium does not.

Experiments with Curare in Addition to the Salts.—We have therefore carried out a few experiments in which convulsions were prevented by the previous administration of curare. We shall illustrate the results by the following two protocols.

EXPERIMENT 15.—Calcium chloride; curare. Male rabbit, 1907 grams. Usual preparation; burette filled with calcium chloride in *M*/1 solution. Curarin injected until spontaneous respiration was abolished; started artificial respiration.

12 m. Infusion begun.

12.11 p. m. Heart not palpable; stopped infusion; 7.5 c.c. ran in; stopped artificial respiration; no convulsions. Thorax opened; occasionally a heart beat.

12.50 p. m. Heart moderately contracted; jaws and legs show beginning stiffness.

1.20 p. m. Jaws locked; neck rigid; legs rigid and extended.

2.45 p. m. Rigor nearly complete, of an extension type. Next day very little softening was present.

Curare, which usually retards the onset of rigor, here exerted no influence whatsoever. Although no convulsions occurred, the rigor developed as early as is usual with such a dose of a molecular solution of calcium chloride. This experiment teaches therefore in the first place that the acceleration of the onset of rigor by the transfusion of calcium salts is not due to an antemortem appearance of convulsions. But it teaches us also that the delaying effect of curare is completely wiped out by the accelerating effect of calcium.

EXPERIMENT 16.—Magnesium chloride; curare. Female rabbit, 1320 grams. The usual preparation; burette filled with magnesium chloride in *M*/1 solution. Curare given until effective; started artificial respiration.

12.35 p. m. Infusion begun; given very slowly.

12.50 p. m. Stopped infusion, 7 c.c. infused; stopped artificial respiration. No convulsions; thorax opened; no heart beats.

2.05 p. m. Everything perfectly soft.

2.45 p. m. Jaws stiff, everything else flaccid.

4.45 p. m. Except in jaws no stiffness in any part, including heart. Animal was not seen until next day at 4 p. m., when the following note was made: Some rigor of legs present, but less than in the calcium chloride rabbit.

In this and in other experiments in which magnesium salts and curare were employed at the same time, the delay in the onset of the rigor was greater than in animals which had either magnesium alone or curare alone. The retardation of rigor mortis after magnesium salts would therefore seem to be in a degree due to some other factor besides the capability of paralyzing nerve and muscle.

Experiments with Magnesium Salts and Strychnine.—That, however, the paralyzing effect of magnesium salts is an essential factor in the delay of rigor can be seen from the following two experiments, in which, besides magnesium salts, strychnine was administered.

EXPERIMENT 17.—Magnesium chloride and strychnine. Female rabbit, 1940 grams. Usual preparation; burette filled with magnesium chloride in *M*/2 solution.

2.27 p. m. Infusion begun; given slowly.

2.35 p. m. 3 c.c. infused so far; respiration slow and labored; started artificial respiration.

2.38 p. m. 9 c.c. infused so far; no corneal reflex; animal limp.

2.40 p. m. Injected intramuscularly 1.5 milligrams strychnine nitrate—more than a minimum fatal dose.

2.54 p. m. Stopped infusion; 18 c.c. infused.

2.55 p. m. Artificial respiration stopped temporarily, heart beating; artificial respiration resumed.

2.58 p. m. Stopped artificial respiration; no convulsions.

3.00 p. m. Animal dead.

3.48 p. m. and 4.25 p. m. All parts perfectly limp.

5.15 p. m. Jaws stiff now; all other parts flaccid.

Animal was not seen until next day at 10.30; then all parts were rigid.

There were no strychnine convulsions; the magnesium salts as well as the artificial respiration inhibited them, and there was apparently the same delay in rigor as seen after the administration of magnesium salts alone.

EXPERIMENT 18.—Magnesium sulphate and strychnine. Female rabbit, 1940 grams. Usual preparations; burette filled with magnesium sulphate in *M*/2 solution.

11.09 a. m. Injected subcutaneously one milligram of strychnine nitrate, 0.5 milligram per kilo being only a toxic dose.

11.16 a. m. Animal hyperesthetic.

11.19 a. m. Convulsions; started artificial respiration and began infusion of magnesium sulphate very slowly.

11.22 a. m. Convulsions stopped; no hyperesthesia.

11.24 a. m. On stopping artificial respiration tremor of chest began again; artificial respiration resumed.

11.35 a. m. No tremor anywhere.

11.42 a. m. Infusion stopped; 30 c.c. ran in; stopped artificial respiration; no convulsions or tremor followed.

11.48 a. m. Heart still beats occasionally.

12.15 a. m. Definite stiffness of hind and front legs.

12.55 a. m. Stiff as a board.

Next day at 5 p. m., there is no beginning of release from rigor.

Although this animal received a very large dose of magnesium sulphate, the rigor was not delayed by it; the onset and development were as much accelerated, as if strychnine alone had been administered. The accelerating effects of a tetanus apparently cannot be perceptibly modified by the subsequent administration of magnesium salts.

We have made a few experiments on the onset of rigor under so-called normal conditions of death, but we shall not enter into

a discussion of these experiments nor draw any conclusions from them. From our own studies and from a study of the literature we believe that every mode of killing employed by various writers, for instance, pithing, hanging (asphyxia), exsanguination, etc., has a specific relation to the onset of rigor and has to be studied separately in a longer series of observations.

Conclusions Drawn from the Infusion Experiments.—Our experiments with the intravenous infusion of calcium and magnesium salts appear to have quite firmly established the following facts. In the first place, it seems quite certain that calcium salts hasten and magnesium salts retard the onset of rigor mortis in skeletal muscles. These effects appear to increase with the quantity of the salts introduced into the circulation; the degree of the molecular concentration in which the solutions are employed seems to be of lesser importance. The accelerating effect of the calcium salts is apparently independent of the state of contraction of the muscles; there is a strong acceleration even when antemortem convulsions are prevented by means of curare. The delay due to magnesium is apparently caused, in the first place, by the paralyzing effect of these salts upon the nervous system and the muscles. Besides the paralyzing effect, the magnesium salts may, however, contain another factor which aids in a smaller degree in the delay of onset of the rigor. The delay following magnesium plus curare seemed to be greater than that following either magnesium alone or curare alone.

Another striking difference, which was frequently observed in these experiments and which deserves to be recorded, consisted in the difference of the type of rigor which followed each of the two kinds of salts. After infusion with calcium salts, especially after larger quantities, the preponderance of extension was very manifest. Even when the animals soon after death were placed in a position in which flexion prevailed, namely, with the head bent forward, the abdomen concave and all the extremities flexed, they were found later in a strong position of opisthotonus: the head strongly drawn backward with a pronounced lordosis and the extremities stretched out. The position of the magnesium animals, on the other hand, was always that in which flexion prevailed, or perhaps more correctly, the animals remained in the position in which they were placed originally.

As to the degree of rigidity which the muscles finally attained, there seemed to be no difference between calcium and magnesium, both animals were at one time or another stiff as a board, only that the maximum stiffness was attained in the magnesium animals much later than in the calcium animals.

The release from rigor appeared as a rule definitely earlier in the calcium animals than in the magnesium animals. There were only a few exceptions in which the rigor in the calcium animals lasted as long or even longer than in the magnesium animals and in these cases the quantity of calcium salts used was greater in proportion than that of the magnesium control. The earlier release from rigor in the calcium animals seems to be due to the earlier development of rigor and occurs in all cases in which the rigor for any reason sets in early (Bierfreund). We have, however, to state that on account of the high temperature of the season in which most of the experiments were carried out not all of the animals were kept until the release from rigor was completed.

The action of the salts upon rigor was caused, at least in the main, by the cations, that is, by the calcium and magnesium parts of the compounds. As regards the anions it is probable that they too exert some effect. Calcium acetate, for instance, seemed to hasten the onset of rigor more than any of the other calcium compounds. Our experiments were in this regard not sufficiently numerous and varying to permit the giving of precise data. We believe, however, that our experiments justify the general statement that the effect which the anions may have is insignificant in comparison with the effects which the cations of the salts exert upon the onset of rigor.

As regards the order in which the several parts of the body enter into rigor, the rigidity of the jaws appeared first in our experiments and usually far ahead of the stiffness of the other parts. The neck was the next part in which stiffness appeared. In a few cases, however, the neck was the last part in which the rigor set in. Of the extremities, in the majority of our experiments, the hind legs became rigid before the front legs. This differs from the so-called Nysten system, according to which the arms become rigid before the legs, For rabbits, however, our observation agrees with those of previous observers(14).

Cutting the sciatic nerves, which is said to retard the development of rigor(39), produced in our experiments no definite results. Sometimes there was a slight retardation of the onset on the side on which the nerve was cut, but nearly as often the retardation was on the opposite side. Furthermore, even in animals with both sciatic nerves intact, sometimes the right and sometimes the left leg became stiff first; the same occurred also in the front legs.

Finally we made incidentally some notes on the onset of rigor in the heart. The observations were not so extensive and precise as those made on the skeletal muscles; but they were sufficiently numerous to permit the general statement that calcium hastens and magnesium retards the development of rigor of the left ventricle. The observations upon the right ventricle were insufficient to warrant any positive statement.

Experiments with Intra-arterial Injections.—In the foregoing experiments with intravenous infusions, the salts, even when given in molecular concentration, reached the tissues in a dilute state and reached them always in company with some blood, which according to von Fürth(26), prevents the coagulation of the muscle plasma and interferes also with the development of muscle rigor. In the experiments which we are now going to record the injections were made into the arteries towards the periphery. In these experiments the muscles of that peripheral part towards which the injections were made, came into intimate contact with a more concentrated solution of the injected salts and probably also without a great admixture of blood.

As mentioned above, such experiments were already made by von Fürth. He tested a large number of substances in mammals as well as in frogs. However, as far as the substances employed in our experiments are concerned, he made only two experiments in frogs, one with calcium chloride and one with calcium nitrate; at least these are all that he records and it is not indicated that more experiments were performed than recorded. Von Fürth has seen no accelerating effects from injections with calcium salts. We have made such injections with calcium and magnesium salts in rabbits and frogs. We shall report first our experiments on rabbits.

Intra-arterial Injections in Rabbits; Method.—The animals had

morphin and ether; laparotomy was made. The abdominal aorta was ligated always beneath the renal arteries, and a cannula was tied in its peripheral end. In some experiments the inferior vena cava also was ligated, in others again the cannula was tied in one of the common iliac arteries. When the cannula was in the aorta, one of the common iliacs was clamped during an injection in order to direct the solution into one limb, using the other as a control. The injection was made with a syringe and usually quite rapidly—not less than six or seven cubic centimeters in a minute. In these experiments calcium chloride only was tested, while of the magnesium salts the chloride and the sulphate were investigated. Only $M/8$ and $M/1$ solutions were used.

In these experiments the animals usually died during the injection or soon after it, even when the inferior vena cava was clamped—a proof of the sufficiency of the collateral circulation. When the injections were made into the aorta, with one common iliac clamped, the tissues of both sides of the lumbar region were seen to be invaded by the liquid and to become blanched. Probably also the lumbar section of the spinal cord came into intimate contact with the solutions, a point which may have to be taken into consideration when analyzing the results.

We shall again illustrate our results with a few greatly abbreviated protocols.

Series I. Intra-arterial Injections into the Hind Legs of Rabbits; M/8 Solutions.

EXPERIMENT 19.—Calcium chloride. Female rabbit, 1350 grams. Preparation as stated above; abdominal aorta ligated. Cannula tied in left common iliac artery.

12.30 p. m. Injected 20 c.c. calcium chloride of $M/8$ solution into left leg.
12.34 p. m. Thorax opened, death; no immediate stiffness.
12.40 p. m. Left leg shows some resistance.
1.30 p. m. Distinct stiffness in left leg, right flaccid.
1.55 p. m. Left leg stiff in all joints, right leg soft.
3.25 p. m. and 4.20 p.m. Left leg like a board, held extended; some resistance in right leg.

In the calcium leg the rigor began about ten minutes after injection and in the control leg not till after three hours.

EXPERIMENT 20.—Magnesium sulphate. Female rabbit, 1450 grams. Usual preparation; cannula in aorta; left iliac clamped.

11.55 a. m. Injected 30 c.c. magnesium sulphate in *M*/8 solution (into right leg). Had slight convulsions after injection.

12.00 m. Thorax opened, heart still beating.

4.00 p. m. Front legs and left hind leg stiff; right leg flaccid.

The magnesium leg was flaccid, while the other parts were in full rigor.

EXPERIMENT 21.—Calcium chloride in one leg and magnesium chloride in the other. Female rabbit, 1120 grams. Usual preparation; cannula in aorta.

11.30 a. m. 13 c.c. *M*/8 magnesium chloride injected into left leg (right iliac clamped).

11.34 a. m. Struggles.

11.35 a. m. 13 c.c. *M*/8 calcium chloride injected into right leg (left iliac clamped). Thorax opened, heart still beats.

11.50 a. m. Right leg (calcium) stiffer than left.

12.40 p. m. Left leg soft; good resistance in right.

3.20 p. m. Right leg very stiff; some stiffness in left.

In this experiment the difference between the calcium and the magnesium was striking enough, but the acceleration of the rigor in the calcium leg and the delay in the magnesium leg were not so striking as in the other experiments in which larger doses were injected.

In these and other experiments of this series the calcium was distinctly accelerating and the magnesium delaying on the onset of rigor, the injected quantity being a noticeable factor. There was no perceptible effect immediately after injection.

Series II. Intra-arterial Injections of M/1 Solutions.

EXPERIMENT 22.—Calcium chloride. Male rabbit, 1410 grams. Usual preparation; cannula in aorta; left common iliac clamped.

10.30 a. m. Injected 30 c.c. *M*/1 solution (into right leg); animal died during injection.

10.33 a. m. Right leg definitely stiff at hip.

10.35 a. m. Right leg stiff at all joints.

11.30 a. m. Right leg very stiff; slight stiffness in left leg as well as in front legs.

This greatly abbreviated protocol shows that in the injected leg the rigor began two or three minutes after injection, and was not followed by any temporary relaxation, while in the other parts of the body the rigor began about one hour after death.

Out of four experiments with calcium chloride, *M*/1, only in one was there a brief relaxation intervening between the primary contraction and the final rigor.

EXPERIMENT 23.—Magnesium sulphate. Female rabbit, 1250 grams. Usual preparation; cannula in aorta; left common iliac artery clamped.

11.35 a. m. Injected (into right leg) 30 c.c. $M/1$ magnesium sulphate; animal died during injection.
11.38 a. m. Definite stiffness of right leg at hip, left leg soft.
12.05 p. m. Both legs soft at all joints.
2.00 p. m. Both hind legs soft; slight stiffness of neck and front legs.
3.30 p.m. Some stiffness at hip in both legs; more on right side.

Three minutes after the injection of magnesium sulphate the injected leg became stiff, but softened soon, to become rigid again. Rigidity occurred even later than in the other parts of the body. The same occurred in two other experiments with $M/1$ magnesium sulphate.

In two experiments with $M/1$ magnesium chloride (30 c.c.) the leg into which the injection was made became stiff soon after the injection, the stiffness developing into complete rigor without an intervening relaxation. The injected leg behaved in these experiments rather like the leg in the majority of the calcium experiments. With regard to the other parts of the body there was no difference in the behavior towards the onset of rigor between the magnesium sulphate and magnesium chloride animals. In both cases the rigor developed in the rest of the body later than in the calcium animals, but not as late as in the animals which received the magnesium salts intravenously.

While in the intravenous injections the degree of concentration of the solutions affected but little the final results, in the intra-arterial injections the degree of concentration was quite an important factor. When the salts were injected in $M/10$ solutions, there was in the intra-arterial injections the same difference between the magnesium and calcium salts as in the intravenous injections, the former uniformly delaying, the latter accelerating the onset of rigor. In either case the cation was the main factor and the anion was of very little importance. It was very different, however, when in the intra-arterial injections the salts were given in $M/1$ solution. Here the calcium chloride as well as both magnesium salts brought on a distinct stiffness almost immediately after injection. In the magnesium sulphate animal the stiffness gave way again, the real rigor developing much later. In most of the calcium animals the early stiffness went over into complete rigor without any temporary relaxation.

The same occurred also in the magnesium chloride animals. The

early stiffness in all these cases was apparently not rigor, but simply a real contraction of the muscles, caused by the stimulation of the concentrated solutions. It was neither a specific calcium nor magnesium effect.

The rapid injection of a $M/1$ solution had the effect of a so-called "salt action," an osmotic action, perhaps. And here we found that the anion is a significant factor, the magnesium chloride acted more like calcium chloride and less like the magnesium sulphate. That in the cases of both chlorides the primary stiffness passed over directly into rigor might have been due to the greater contraction which they have primarily produced and finds its analogue in the direct transition of a strychnine tetanus into rigor, the development of which, as we found above, magnesium salts cannot suppress or delay. The same conditions probably obtain in all cases of so-called chemical rigor (see von Fürth), for instance, after injection of chloroform, ether, caffein, sodium mono-brom-acetate, quinine, antipyrin, etc.; these substances cause primarily strong contractions, which hasten the onset of rigor and into which the contractions pass without any intervening relaxation. These chemical rigors are work-rigors (Arbeitsstarre) as Santesson(40) properly terms them. The rigor which follows nearly immediately after the injection of calcium and magnesium chloride belongs therefore to the group of work-rigors and two stages must be distinguished: the stage of simple tonic contraction and the stage of actual rigor. If the tonic contraction is moderate as in most of the cases when magnesium sulphate is injected in $M/1$ solution a longer or shorter intermission of relaxation follows between the stage of contraction and that of the real rigor.

From the production of this work-rigor must be distinguished the accelerating effect of the calcium salts upon the onset of rigor in the cases where only $M/8$ solutions were employed intra-arterially or when even $M/1$ solutions were injected intravenously. In these cases at least twenty or thirty minutes passed before the onset of rigor which was never preceded by a preliminary contraction. The effect here is apparently purely a chemical one confined to the cation calcium and is never caused by any anion combined with magnesium.

In the intra-arterial injections into one of the legs the effect of

the salts upon the other parts of the body was not so well pronounced as in the intravenous injections, although the entrance of the solution into the general circulation was prompt enough to prove fatal to the animal. The premature killing of the heart caused by the rapidity of the injection prevented the salts from satisfactorily reaching and saturating the tissues so as to bring them under the same degree of influence as in the slow intravenous injections with a prolonged efficient circulation.

The subcutaneous injections which were made on rabbits, guinea-pigs and rats were not numerous and we shall not speak of them in detail. It will suffice to say that the results of these observations agree with the main results obtained by the intravascular method, namely that calcium hastens and magnesium retards the onset of rigor.

Experiments on Frogs.—The injections were made in the lymph sacs and through the aorta. The rigor in frogs sets in very late after death, sometimes even as late as thirty-six hours. The interval varies with the species of frog, with their state of nutrition, and with the season of the year. Our studies were made on *Rana esculenta,* in the early spring and at the end of the summer, and some of the animals were in a rather poor state of nutrition. In all of the controls the rigor never appeared within the first twenty-four hours. As out of every twenty-four hours the animals can be under direct consecutive observation not more than ten, it is frequently impossible to note the onset of rigor if no artificial means are employed to hasten it. We made only two sets of experiments in which the animals received the injection of the salt solutions in the lymph sacs and then left them without further interference. Another drawback in the experiment is that if both frogs received the injection simultaneously, the calcium animal survived the magnesium animal by many hours, in fact, the exact time of their death could not be noted in our experiments; they were found dead in the morning. But even so, the calcium animals of our experiments were found already quite rigid, while the magnesium animals, although they died shortly after the injection in the middle of the previous day, were either still completely soft or showed only slight stiffness. We may therefore conclude even from these few, incom-

plete experiments that calcium hastens the onset of rigor in frogs. As to the delaying effect of magnesium salts these experiments gave no positive evidence.

In the experiments with transfusions through the aorta calcium chloride, magnesium chloride, magnesium sulphate and also sodium chloride were tested in $M/1$, $M/2$ and $M/10$ solutions. In the infusions with $M/1$ solutions all animals became very stiff in the course or at the end of the infusion and remained so for several days, while the control animals became rigid on the second day and were relaxed again about twenty-four hours later. The brain was destroyed in all animals. The cord was destroyed in one set and left intact in another set. In the animals with destroyed cords the rigidity was a little less strong than in the animals with cord intact. The early and persistent rigidity occurred not only in the calcium frog, but also in the animals which received magnesium sulphate, magnesium chloride or sodium chloride in $M/1$ solutions.

A similar effect was observed when the transfusions were made with $M/2$ solutions; the onset of rigidity occurred immediately after infusion and persisted for a few days and there was also the same difference in the degree of stiffness between the animals with destroyed cord and those with cords intact.

In the above mentioned two experiments of von Fürth, one with calcium chloride and one with calcium nitrate, ten cubic centimeters of a ten per cent. solution were injected through the aorta of frogs whose cord was destroyed. A ten per cent. solution of calcium chloride is nearly equal to the $M/2$ solutions which we have, among others, employed. Von Fürth thus summarizes his observations: no rigor over night—"Ueber Nacht keine Starre." On account of the divergence of our results with those of von Fürth we shall give an abbreviated protocol of one set of such experiments.

EXPERIMENT 23.—Calcium chloride infusions in the aorta of frogs. Three medium-sized frogs. Frog 1, cord intact. Frog 2, cord destroyed. Frog 3, control. Aorta connected with burette; sinus opened; all three frogs had 30 c.c. 0.6 per cent. sodium chloride solution ran through to wash out the blood. Frogs 1 and 2 had then a transfusion with 12 c.c. calcium chloride in $M/2$ solution.

3.00 p. m. Finished transfusion. Frog 1 stiff, Frog 2 moderately stiff, and Frog 3 flaccid.

4.30 p. m. No relaxation in Frogs 1 and 2; control still soft.

Next day at 12.00 m. and 6.00 p. m., Frog 1 is very stiff, Frog 2 stiff, but less than Frog 1; Frog 3, control, is moderately stiff.

There was no doubt as to the early and persistent rigor of Frog 2—comparable with von Fürth's experiment—although the rigidity was somewhat less than in the frog with intact cord. Could the difference between our experiments and that of von Fürth be due to the difference of the character of frogs? Von Fürth experimented on *Rana temporaria* and we used *Rana esculenta.* It could not be due to the previous washing out with a sodium chloride solution, as we had the same results without such a previous washing. We must again point out that no matter how thorough and extensive von Fürth's experiments otherwise were, on the particular point in question there were only two experiments and they were entirely insufficient to controvert our results which were, moreover, in complete harmony with the results which we obtained in mammals. This agreement was not disturbed by the fact that in frogs even after infusion of magnesium sulphate there was no relaxation of the primary stiffness, since the dose which we employed in frogs, considering the small size of these animals, was in proportion much greater than that employed in mammals.

The fact that in the frogs with intact cord the rigor is stronger than in frogs with destroyed cord has its explanation in that the muscle tissue alone is stimulated in the latter by the concentrated salt solutions, while, when the cord is intact, the simultaneous stimulation of the nervous mechanism increases the motor response of the muscles, which in turn leads to a stronger rigor. The relation of the intensity of the rigor to the stimulating effect of the salts upon the cord reminds one of the strong rigor which follows strychnine poisoning.

In the frogs which received infusions with $M/10$ solutions of the salt the rigor developed late in all animals, and it was difficult to decide whether there was any acceleration or delay in its onset in case of one or the other of the salts.

However, the experiments with transfusions of $M/10$ solutions, as well as experiments in which the animals received in their lymph sacs comparatively large doses of $M/1$ solutions of the salts (about four cubic centimeters for each frog), brought out distinct results

when the posterior half of the body of the frog not skinned was submersed in a sodium chloride bath, kept at a temperature of about 38° C. This method was first successfully employed by Langendorff and Gerlach(41), and later by Nagel(18). Of course, the rigor which sets in here quite early is a heat rigor. But here again the development of the rigor in the calcium animals was far ahead of that of any of the other frogs, which received either one of the magnesium salts or sodium chloride, or were simply control animals. The lower extremities of the calcium frogs pass more or less rapidly through a stage of flexion and abduction into a terminal stage of extreme extension mostly combined with abduction. In the magnesium animals the rigor appears only a little later than in the control animal and the rigid legs remain mostly in a flexed state.

These observations demonstrate that heat rigor also is greatly accelerated by the presence of calcium in the muscles of the animals. The delaying effect of magnesium is not safely established in this line of experimentation.

The experiments on frogs have confirmed the fact that calcium hastens the onset of rigor mortis, and favors also the development of heat rigor. They have also shown that concentrated solutions of calcium and magnesium salts in bloodless animals call forth an immediate contraction of the muscles which passes then without an intermediate stage into the real rigor mortis. As to the delaying effect of magnesium, the experiments on frogs brought no positive results, the failure being essentially due to the late onset of the normal rigor in the animals.

The experiments reported in the foregoing pages have shown that the relations of calcium and magnesium salts to rigor run in opposite directions, that is, calcium hastens and magnesium retards the onset of rigor. We suggested at the outset that if the contraction theory of rigor is correct, both salts ought to retard the development of rigor, that is, on the assumption that both salts exercise inhibitory effects. Can our results be considered as contributing towards a decision between the two theories, namely as being against the contraction theory and therefore in favor of the chemical theory of rigor, and can we assume that the acceleration and retardation of the rigor in our cases have their last cause in the facts established

by von Fürth that calcium salts hasten the clotting of myogen plasma and magnesium salts do not hasten it? It sounds plausible. But there is again some discrepancy between certain points in these facts and certain details in our observations. According to von Fürth(42) magnesium nitrate hastens the coagulation of the muscle plasma, while in our experiments magnesium nitrate has retarded the onset of rigor longer than any of the magnesium salts. Besides, the argument that if the contraction theory be correct, the effects of calcium and magnesium salts ought to be similar, is based, as stated above, on the assumption of the similarity of both salts with regard to their inhibitory effects. Very recent experiments, however, have demonstrated to us that the foundation of this assumption is, to say the least, insecure.

The safer course, therefore, would be not to enter for the present into a discussion of the availability of the facts revealed in this paper in support of one or the other of the theories of rigor.

SUMMARY.

Calcium salts hasten and magnesium salts retard the development of rigor mortis, that is, when these salts are administered subcutaneously or intravenously.

When injected intra-arterially, concentrated solutions of both kinds of salts cause nearly an immediate onset of a strong stiffness of the muscles which is apparently a contraction, brought on by a stimulation caused by these salts and due to osmosis. This contraction, if strong, passes over without a relaxation into a real rigor. This form of rigor may be classed as work-rigor (Arbeitsstarre).

In animals, at least in frogs, with intact cords, the early contraction and the following rigor are stronger than in animals with destroyed cord.

If $M/8$ solutions—nearly equimolecular to "physiological" solutions of sodium chloride—are used, even when injected intra-arterially, calcium salts hasten and magnesium salts retard the onset of rigor.

The hastening and retardation in this case as well as in the cases of subcutaneous and intravenous injections, are ion effects and essentially due to the cations, calcium and magnesium.

In the rigor hastened by calcium the effects of the extensor muscles mostly prevail; in the rigor following magnesium injection, on the other hand, either the flexor muscles prevail or the muscles become stiff in the original position of the animal at death.

There seems to be no difference in the degree of stiffness in the final rigor, only the onset and development of the rigor is hastened in the case of the one salt and retarded in the other.

Calcium hastens also the development of heat rigor. No positive facts were obtained with regard to the effect of magnesium upon heat vigor.

Calcium also hastens and magnesium retards the onset of rigor in the left ventricle of the heart. No definite data were gathered with regard to the effects of these salts upon the right ventricle.

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A FATAL ANÆMIA WITH ENORMOUS NUMBERS OF CIRCULATING PHAGOCYTES.[1]

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Phagocytosis of red cells, and to a lesser extent of white cells, has long been known to occur within the blood-making organs of the body in health and to a greater degree in disease. The interest of the present case is that it exemplifies the occurrence of extensive phagocytosis in the peripheral blood, involving a destruction of red cells so rapid that it may well have been the cause of the fatal anæmia which ensued. As to the cause of this phagocytosis I am altogether in doubt, but since it involved every type of leucocyte in the blood, it seems reasonable to attribute it to the presence of autohæm-opsonins in the serum. Details of the case are as follows:

Clinical History.—The patient was a male, Russian Jew, twenty-seven years old. He had resided one year in Boston. His family history, past history and habits were good. The present illness was from five to six months in duration. The first symptoms were palpitation and dyspnœa; five or six weeks later his feet began to swell and he coughed much, especially when lying on the right side. Examination April 20, 1907, showed a well-developed and nourished man, cyanotic and dyspnœic on the slightest exertion. The fingers were markedly clubbed.

Heart's apex was in the fifth space, one and a half inches outside the nipple line; the right border was just outside the edge of the sternum on the right. Action of the heart was regular. There was a systolic murmur at the apex and a diastolic murmur loudest at the third left costal cartilage; a systolic murmur was heard along the right side of sternum, opposite the first, second, and third ribs. The apex systolic murmur was transmitted along the ribs and also heard in the back at the angle of the left scapula. The pulmonary second sound was accentuated. Corrigan pulse was present.

The chest showed inspiratory retraction of the intercostal spaces especially near the right nipple. There was noisy breathing over most of the chest in front and over the upper part behind. Scattered moist rales were most numerous below; in the upper part of the chest were whistling rales. At the bases of both lungs posteriorly the breath sounds were nearly absent (especially on the left) and there was dulness on percussion with diminished voice sounds and fremitus.

[1] Received for publication, November 4, 1907.

The abdomen was distended and tympanitic; the liver dulness extended from a point about an inch below the rib margin to the fifth rib. The abdomen was too distended to allow palpation of the liver or spleen. No fluid was found. The patient complained of much tenderness and pain in the splenic region and a dragging sensation. The legs showed œdema extending to just below the knees. The reflexes were normal. A few enlarged glands were palpable in the groins. The patient lived thirty-four days after the date of this examination. Œdema of the extremities with double hydrothorax, great cyanosis, jugular pulsation and Cheyne-Stokes breathing gradually developed. The urine showed the usual evidences of renal congestion. The anæmia steadily progressed. A small pharyngeal abscess was opened May 8 and found to contain many polynuclear and very few mononuclear leucocytes. A blood culture was taken with negative results.

May 24 the patient died.

Obviously the patient suffered and died from aortic and mitral disease with anæmia. But the chief interest in the case is centered in the blood.

The Blood.—With the exception of two weeks, when the patient was in the Massachusetts General Hospital,[2] he was under my constant observation from March 1, 1907, to the day of his death, May 24, 1907. Cover-glass smears were taken daily and counts of the red and white corpuscles were made at various times as shown by one of the accompanying charts, which shows a very marked and increasing leucocytosis with anæmia. An early study of the blood with the warm stage revealed the fact that there was active phagocytosis of red and white cells not only by the "large lymphocytes," but also by the polynuclear cells and by every variety of leucocyte known in normal blood. The findings suggested a careful and thorough examination of the stained smears which confirmed the warm stage findings. In this case not only were the usual blood cells (polynuclear leucocytes, lymphocytes, eosinophiles and mast cells) engaged in phagocytosis, but also the myelocyte and another type of cell, similar to that which has been described as a "plasma cell."

Warm Stage Findings.—With the warm stage the "lymphocytes" appeared to be very sensitive, responding immediately to increased heat before one would suppose that the stimuli had time to reach them. Striking the slide with a pencil would disturb the cells and cause them to draw in their processes.

[2] A report of this case has been published by Dr. W. B. Bartlett, House officer at the Massachusetts General Hospital (*Boston Medical and Surgical Journal*, 1907, clvi, 629).

An attempt to stain the cells on the warm stage by putting the stain on the slide at the edge of the cover-glass so that it would run under the cover-glass, stopped all motion in the leucocytes and the red corpuscles in their rush across the field easily broke off the processes of the "lymphocytes."

I. *The Capture and Destruction of Cells by "Lymphocytes."*—I found in all the warm stage preparations many round, mononuclear, non-granular cells which on the cold slide were of the size of a lymphocyte either of the large or of the small variety. I shall call them "lymphocytes" without meaning thereby to suggest any theory of their origin.

1. *Changes in the Cell and in the Nucleus.*—On reacting to heat, the lymphocytes *spread out* so as to attain sometimes a great size—almost filling the field of an oil immersion lens with No. 3 eye-piece and processes of protoplasm came out of them, one going over three fields of the oil immersion lens (No. 3 eye-piece). The structure of the nucleus was not noticed until after the cell began to enlarge; then the nucleus also enlarged rapidly, but after it once became enlarged the nucleus rarely went back to its former size and compactness, no matter what changes took place in the protoplasm, not even when a shock was transmitted to the cell which caused it to pull in its processes and to contract its protoplasm closely around the enlarged nucleus. (Plate IV, Fig. 1, *c*, shows a lymphocyte whose protoplasm has returned to the former size while the nucleus still remains enlarged.) Some of the small lymphocytes enlarged but little under the influence of heat and captured only such cells as were in immediate contact with them.

During phagocytosis the *form of the cell* changed rapidly and continuously, new processes going out, those already out changing their position, inclusions being destroyed or more being taken in,—all during the few seconds necessary to make a rough sketch. If the smears were thick, the processes would start out as thin streams, widening when some distance away from the cell. Often the cell nucleus would break and large pieces of it would be carried out into one or more of the processes. (See Plate VII, Fig. 4, and Plate X, Fig. 1, where pieces of the nucleus can be seen in the processes of the cells.) *Later the nuclear matter was sometimes drawn*

back into the cell, which was then multinuclear. With the recognition of this fact it is perhaps unwise to speak of these cells any longer as lymphocytes, since lymphocytes have always been defined as mononuclear. But the mononuclear lymphocyte, as we ordinarily see and define it, is in fact the *resting* lymphocyte. When it is *active,* as in some cases of lymphatic leucæmia and in this case, the nuclear material is certainly divided. In view of the staining reactions of both the nucleus and the protoplasm and of the transition stages recognizable between the classic lymphocyte and the most extreme modification, the huge, multinuclear, many-tailed cells of this patient's blood — it seems best still to retain the old term "lymphocyte."

2. *The Process of Capture.*—Both red and white cells were captured and destroyed. Two methods of capture were observed. In the first a process would emerge and envelop cells immediately adjacent. In the second a process would reach out for some distance, turn as if intending to "round up" a cell lying free in its concavity, then suddenly turn again (making a double curve) and envelop cells that had floated against its convex side while it was making its first turn. Exactly this order or procedure was followed countless times and by many different cells.

3. *The Process of Destroying Leucocytes.*—The inclusions were sometimes destroyed within the processes, sometimes drawn back into the body of the cell and destroyed there. Inclusions were not always immediately destroyed. A phagocytic cell would sometimes: (*a*) destroy one inclusion at a time, or (*b*) take in several before destroying any, or (*c*) destroy one while it was capturing others.

The white cells were destroyed by lymphocytes in two ways:

(*a*) *Mechanical.*—The protoplasm of the phagocytic lymphocyte would repeatedly knead and squeeze the cells until they were broken up and their pieces scattered through the body of the phagocyte. When a polynuclear neutrophile was ingested and compressed, its nucleus always became spherical and either remained so or was later broken up into smaller spheres (Plate III, Figs. 3, *a,* and 6, *b,* and Plate IV. Fig. 4, *b*). The crushing, kneading motions went on with machine-like regularity and approximately at a steady rate.

(*b*) *Chemical* (*perhaps Enzymic*).—The captured cell sometimes

appeared to fade away, its nucleus breaking up or dissolving *in situ,* while the granules of the protoplasm gradually disappeared until nothing was left of the inclusion but a shell or "cloudy" spot in the phagocyte at the point where the captive had been (see Plate VIII, Figs. 4 and 5). The mechanical and chemical destruction of white cells by lymphocytes are shown in Plates VII and VIII.

A study of the stained specimens showed that when a polynuclear cell was well advanced in the process of being destroyed by a phagocyte (whether the phagocyte was a polynuclear cell, a lymphocyte, or an eosinophile) the nucleus and protoplasm of the captive took, with Wright's modification of Leishman's stain, a decided greenish blue color. Occasionally the nucleus but not the protoplasm was so stained.

Occasionally when the cells were closely massed, a change was found in the staining reaction of a polynuclear neutrophile which had not been ingested but was *merely in close contact* with a phagocytic cell. The change in color was the same as that noted in the polynuclear cells after having been acted upon by the cells which had captured them.

4. *Destruction of Red Cells after Phagocytosis.*—Red corpuscles were destroyed on a larger scale (and more rapidly) than were the white corpuscles. A process of the lymphocyte would often "round up" and take in as many as six erythrocytes at a time. When once engulfed the red cells were next compressed into small yellow dots or strings and finally disappeared.

5. *Escape of Captives.*—Occasionally a red cell was seen to escape after being considerably crushed, when it would resume at once its normal shape, showing the wonderful elasticity of red corpuscles.

Some of the polynuclear cells were also seen to escape after they had been crushed until the nucleus was broken up or had become circular (Plate III, Figs. 3, *a,* and 6, *b;* Plate IV, Fig. 4, *b*). Such cells showed no amœboid motion after getting free and the nuclei had lost their structure and had hyaline appearance. Thus damaged a polynuclear leucocyte came to resemble a nucleated red corpuscle in some respects but the neutrophilic granules of the polynuclear cell were still apparent.

6. *Rate of Destruction.*—One lymphocyte was seen to destroy

fifteen red corpuscles in half an hour; another destroyed twenty-seven red cells and fourteen polynuclear cells in an hour, and one watched for three hours was seen to engulf and wholly or partially destroy sixty-seven red cells and twenty-four polynuclear cells. At the end of that time the lymphocyte was still active.

Two lymphocytes were often seen engaged in destroying one captive and occasionally a cell would be seen engulfing a cell which itself had engulfed others (Plate X, Fig. 3).

II. *Phagocytosis by Polynuclear Neutrophilic Leucocytes.*—The process is quite different from that seen in mononuclear cells.

1. The polynuclear cell sends out one or more very slender string-like processes, which appear to arise from the nucleus. No matter how far these strings go out (and they sometimes traverse several microscopic fields, see Plate II, Fig. 4), little or no diminution is apparent in the size of the nucleus or in the protoplasm of the cell whence they issue. These strings next attach themselves to other cells (either red or white corpuscles) by hooking an end into the protoplasm of the captive, or by spreading out and fastening over it like the fingers of a hand.

2. Sometimes the phagocytic cell next moves away, dragging its captive along for some distance before pulling it in. Then after a pause, the string shortens and apparently recedes into the nucleus whence it came, so that the captive is gradually drawn nearer to its captor. The force exerted by the action of these strings is suggested in Plate II, Fig. 2, where a lymphocyte is seen to be pulled out of shape after being hooked by the string-like process of a polynuclear leucocyte. Often a string is seen to fasten its end in a red cell and pull it out of shape until the red has the appearance of a crenated cell.

3. When captor and captive have been brought into closer contact by the action of the "string," the protoplasm of the captor "flows" around the captive, occasionally not taking in the whole of it but nipping off a piece. (See Plate IV, Fig. 2, *b*, where a piece of the red corpuscle is nipped in this way.) In many cases a polynuclear cell takes in only a portion of a lymphocyte, owing to the great size of the latter. The polynuclear cells are always limited in stretching power (about thirty-four microns) and often seem as if they

would split, owing to the large size of the captives they try to ingest. (See Plate IV, Fig. 1, *a*, where the polynuclear cell has taken in the protoplasm of a lymphocyte.)

Effects on the Cells Ingested by Polynuclear Neutrophiles.—After a cell has been engulfed by a polynuclear leucocyte, there is no evidence of the mechanical compression so commonly exerted by phagocytic lymphocytes. Sometimes the included cells seem to be:

1. Destroyed by a chemical process; parts of the nuclei disappear, and the protoplasm "fades."

2. Sometimes the protoplasm of the captives seems to merge with that of the capturing cell, though the nuclei of the captives remain like islands, unchanged, in the protoplasm of the captor. This process seems to occur especially when the polynuclear neutrophile is itself ingested by another cell of the same type. After this last process has been gone through, the capturing cell appears to be larger than formerly.

3. The red corpuscles ingested appear to "fade," their edges first becoming dim (Plate IV, Fig. 6, *b*). They are not destroyed as rapidly as are the white cells. The lymphocytes, on the other hand, destroy the red cells more rapidly than the whites.

Possible Relation of the Lymphocytes and Polynuclear Neutrophiles to Blood-Plates.—The blood-plates were enormously increased in all the specimens of blood taken in this case. In preparations studied on the warm stage I noticed that the polynuclear cells and lymphocytes while in active amœboid motion were constantly losing small pieces of their protoplasm. These loose fragments bore very close morphological likeness to the blood-plates, as seen in film specimens taken at the same time, and a careful search of the slide failed to reveal any blood-plates differing from these broken-off pieces of protoplasm.

Amœboid Locomotion of the Polynuclear Leucocytes.—Two parts could be distinguished in the polynuclear leucocytes—a clear portion and a granular one (suggesting the endosarc and ectosarc of the ordinary pond amœba). The polynuclear cells were actively amœboid; sending out thick, rounded processes from the protoplasm, wide or narrow, for long or short distances and drawing up the rest of the cell towards the process (like a baby creeping) were the usual amœboid motions.

Presence of Atypical Leucocytes ("Plasma Cells").—Among the leucocytes active and phagocytic in this blood were seen some which corresponded fairly well to one of the types described by some writers as plasma cells. They resembled in general the large lymphocytes, but their protoplasm was intensely basophilic (bright blue with Wright's stain) and contained large unstained granules which could be identified overlying the red or white cells ingested (Plate V, Fig. 4). (I have also heard this cell described as vacuolated.) Mitosis was found in this cell as in all the other cell types of this blood (Plate V, Fig. 6).

I have several times found similar plasma cells in leucæmic blood and in one case (lymphoid leucæmia) I found it containing inclusions.

Relation of Phagocytosis to the Anæmia Seen in This Case.—In less than three months the patient's red cells fell from 5,120,000 to 1,520,000 per cubic centimeter. This progressive and fatal anæmia (shown in the accompanying chart) might very possibly be explained as a result of the great and continuous destruction of red corpuscles by the white cells. (Plate IX suggests the number destroyed.) Since one cell destroyed twenty-seven red corpuscles in an hour on the warm stage at 98° F., when the leucocyte count was 100,000 per cubic millimeter, if *all* were engaged in the process of destroying red cells *at the same rate* they could destroy all the red cells in the body within two hours. Hence if even a small proportion of the leucocytes were phagocytic, even if their average rate of ingesting cells was considerably lower than that which I observed on the warm stage at 98° F., the amount of work called for on the part of the blood-making organs would be enormous and probably overwhelming. Hence anæmia would result.

The Type of Anæmia.—The type of anæmia seemed to vary curiously from day to day; at one time the specimens suggested a secondary anæmia with low color index, small corpuscles and normoblasts; at another period I found appearances resembling pernicious anæmia with a high color index, large red cells and megaloblasts predominating over the normoblasts. March 1, when the red count was high and the white cells showed but little phagocytosis, compared with later smears, an occasional large nucleated red cell was seen.

Variations in the Counts of the Blood Taken from Different Parts of the Body.—May 17, smears taken from each ear showed large-sized red corpuscles with a high color index, polychromatophilic cells (rarely a stippled one) and erythroblasts, but the latter were decidedly more numerous in the smear from the left ear,—eleven megaloblasts and two normoblasts in a differential of two hundred white cells, while the smear from the right ear showed only two megaloblasts and one normoblast. Puzzled by this excess of erythroblasts from the left ear over those from the right, I made a red count from each ear with the following results.

Right ear: 2,740,000 per cubic millimeter (only three erythroblasts).
Left ear: 1,430,000 per cubic millimeter (thirteen erythroblasts).

I tried this experiment twice on the same day with similar results.

The number of leucocytes in specimens of blood taken from the ears, face, shoulders, arms, legs and toes varied greatly at all times. Sometimes successive drops of blood taken from a single puncture showed very different counts. The amounts of phagocytic activity also varied from day to day but phagocytic cells were always to be found in smears from any part of the body and at no time was any great search required to find cells with inclusions.

Possible Reasons for These Differences.—The variation in the number of white cells in the peripheral blood from various parts of the body and even in successive drops from the same puncture (as shown both by white counts and smear-preparations) might be partially explained by the fact that the cells showed a tendency to clump and adhere in masses. This was seen on the warm stage, in the blood counting chamber and in the stained film-specimens. As many as fifty cells were sometimes found massed together. But though this agglutination might explain in part differences in the white counts, it does not help to explain the variation in the red counts, for no such agglutination was noted among the red cells.

The variations in the total leucocyte curve are more reasonably explained, I think, as due chiefly to the *variations in the amount of phagocytosis of leucocytes by leucocytes.*

The *larger* number of the actively phagocytic lymphocytes present in the blood from the left ear (see Table I showing differential counts) might likewise explain the *smaller* number of red cells in the same specimen.

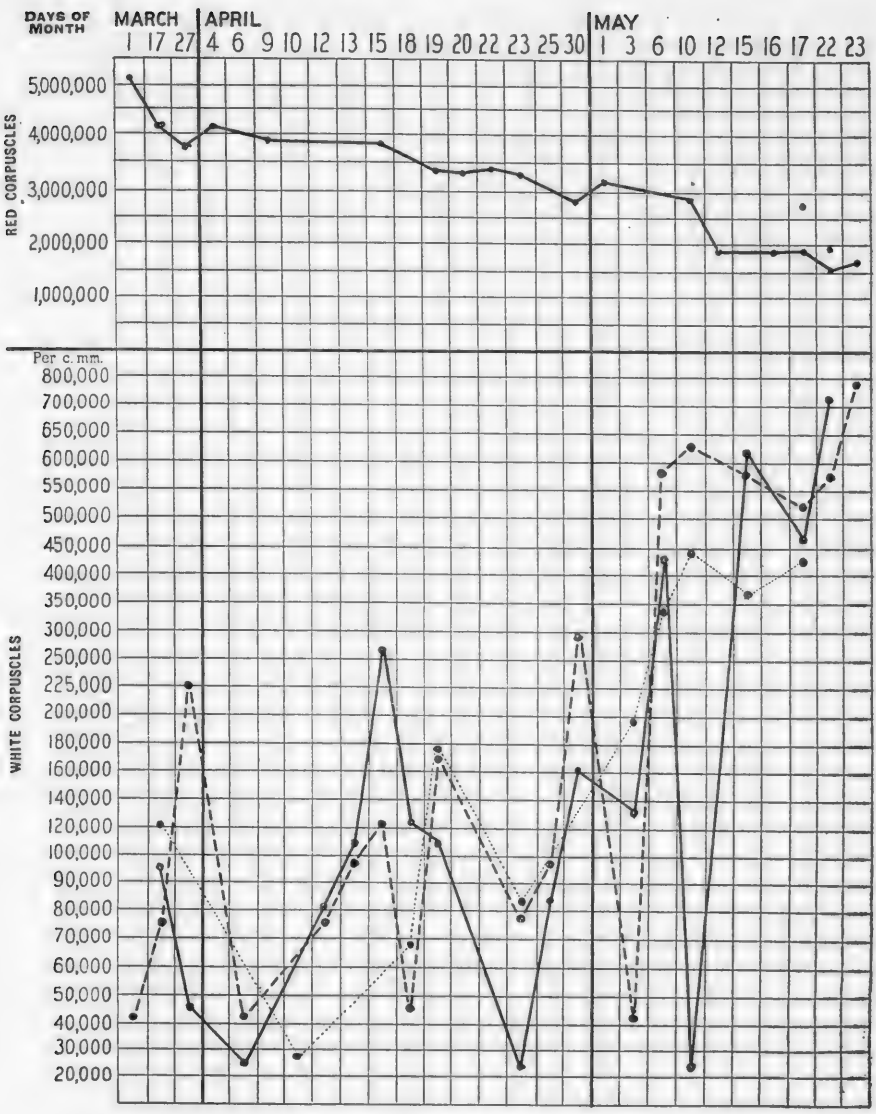


CHART I. In the lower portion of the chart the solid line —— = leucocytes from right ear; the broken line - - - - = leucocytes from left ear; the dotted line .... = leucocytes from extremities.

I have often seen variations in the differential counts in different smears taken from one puncture in cases of leucæmia, both lymphoid and myeloid. Whether there are similar variations in the leucocyte counts made with the Thoma-Zeiss apparatus, I do not know; but I hope to work this point out later.

The Course of the Leucocyte Curves in This Case.—In Charts I and II, and Table I, I have collected the data regarding the leucocytes in this case. In Chart I, the counts from the right ear, from the left and from the extremities are separately plotted. This chart

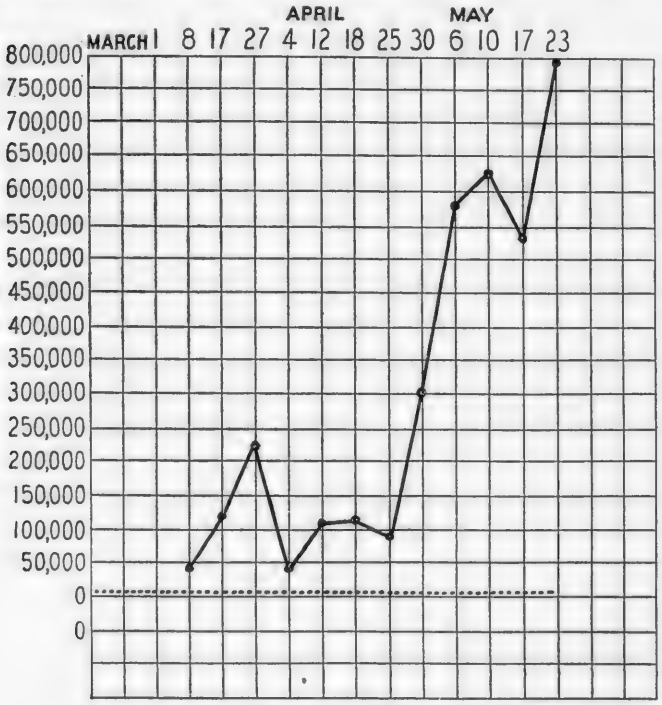


CHART II. Highest counts taken at (nearly) weekly intervals.

shows that there was on the whole an increase in the circulating leucocytes from the 43,000 found March 1 to the 700,000 present May 22. It will be noticed that the last three counts from the ears (May 15, 17 and 22) all show more than 450,000 leucocytes.

In Chart II, I have taken the highest counts only as estimated in (approximately) weekly examinations and plotted the chart accordingly on the theory that the high peripheral counts are more likely to be representative of the whole blood than are the low counts, because the low counts are likely to result in part from massing of the leucocytes or from the phagocytosis of leucocytes by leucocytes.

TABLE I.

	Differential Count of 200 White Corpuscles.							Found in Count of 200 White Corpuscles.	
Dates.	Polynuclear Cells.	Large Lymphocytes.	Small Lymphocytes.	Eosinophiles.	Mast Cells.	Myelocytes	Plasma Cells.	Megaloblasts.	Normoblasts.
March 1	63.	19.	11.	3.	2.5		1.5	1	
March 17	56.5	13.	29.5	1.					
March 22	64.5	17.	18.5						
March 30	56.5	24.5	11.5	5.	2.5				
April 6	64.	22.	14.						
April 9	63.	30.	1.5	2.5	2.		1.		
April 19	77.5	21.5		5.	.5				
April 23 (Rt.)	77.5	22.					.5		
" " (Lft.)	46.5	32.	21.5						1
April 30 (Rt.)	68.	27.5	2.	2.	.5				
" " (Lft.)	47.	32.	21.						
May 5	86.	7.5	1.				5.5	3	1
May 6 (Rt.)	62.	22.	1.5	1.					
" " (Rt.)	69.	26.	3.		2.				
" " (Lft.)	66.	27.5		2 5	2.		2.		
" " (Lft.)	68.5	28.	5.	1.5	1.5		.5		
May 7	68.	25.5		1.5	3.	.5	1.5	1	1
May 15 (Rt.)	66.5	25.5	3.5	1.5	1.	1.	1.	3	1
" " (Lft.)	48.5	43.	4.5	.5	.5	2.	1.	6	2
" " (Foot)	51.	47.5	1.5					1	1
May 16 (Rt,)	61.5	33.	4.	1.	.5				
" " (Lft.)	48.	48.5	2.5			.5	.5	4	2
May 17 (Rt.)	63.	35.	1.			.5	.5	2	1
" " (Lft.)	58.	38.5		.5	1.		2.	11	2
" " (Finger)	37.	54.5		5.5	2.5		.5	1	1
May 23 (Rt.)	65.5	32.5	5.		1.5			1	
" " (Lft.)	64.5	32.	2.5	.5	.5			1	1

The differential counts (see Table I) are difficult to interpret. Except on one day, smears taken from the right ear showed always a much higher percentage of polynuclear cells than did the smears from the left ear, while the mononuclear cells were correspondingly more numerous on the left than on the right side. The percentage and absolute number of large phagocytic "lymphocytes" increased as the case progressed and the number of small lymphocytes diminished.

Eosinophiles varied from zero to	23,000 per cu.mm.	(May 17.)
Mast cells varied from zero to	11,600 per cu.mm.	(May 6.)
Myelocytes varied from zero to	11,500 per cu.mm.	(May 5.)
"Plasma cells" varied from zero to	10,400 per cu.mm.	(May 17.)
Megaloblasts varied from zero to	28,000 per cu.mm.	(May 17.)

Effect of the Patient's Blood in Stimulating Phagocytosis in Other Human Blood.—One platinum loop full of the patient's blood was mixed with about ten loops of blood taken from a healthy adult, whose blood had previously been examined and found to be free from phagocytosis. The two were mixed upon a cold glass slide, covered with two cover slips and put upon the warm stage at 98° F. On immediate examination very little phagocytosis was observable and the experiment was at first considered negative, but an hour later a very much larger number of cells were found to be phagocytic and finally all the mononuclear cells of the mixed blood showed phagocytic activity.

Since not all of these mononuclear cells can have come from the patient's blood a part at least of the phagocytic activity of the mixed blood must be attributed to a stimulus exerted upon the normal leucocytes by the presence of substances (perhaps opsonins) in the patient's serum.

Animal Experiment.—In the last week of April, 1907, five drops of the patient's blood was diluted with about a drachm of normal salt solution and introduced into the subcutaneous tissue of a guinea-pig's abdomen. The blood of this animal had previously been examined carefully and found to contain no evidences of phagocytosis.

About two weeks after the introduction of the patient's blood into the guinea-pig specimens taken from the animal's ear vein showed that approximately two thirds of all the mononuclear cells were engaged in phagocytosis. The cells included by the phagocytes were mostly mononuclear leucocytes, but one polynuclear cell and a few red corpuscles were also ingested.

Two weeks after this or four weeks from the time when the patient's blood was first introduced into the guinea-pig, the blood from the animal's ear vein showed that phagocytosis was still going on, though only about one half as many cells as formerly were now found to contain inclusions.

Two months after this, or three months from the time of the original injection, the animal's blood was again examined. Phagocytosis had then almost entirely ceased. The animal was killed and autopsied but nothing abnormal found.

The Extraordinary Vitality of the Phagocytic Leucocytes in This Case.—When removed from the body the leucocytes from this case preserved their powers of motion and phagocytosis for weeks and even for months as is shown by the following experiments:

1. April 20, 1907.—A drop of this patient's blood was drawn into the Thoma Zeiss pipette and then diluted with 0.5 per cent. acetic acid. May 10 an examination of a drop of this diluted blood (which had remained for nearly three weeks in the bulb of the pipette) showed that the leucocytes were still amœboid, phagocytic and able to send out processes reaching across two squares of the counting chamber.

2. April 22.—Examination of the patient's blood showed 3,290,000 red cells and 6,800 leucocytes per cubic millimeter. The diluted blood was then left (sealed up) in the pipettes. May 18 (or nearly a month later) the contents of the pipettes was again examined with the following results: Red cells 9,000, leucocytes 5,400.

There was then added to the remaining portion of the diluted blood a small amount of normal blood, so that the figures were increased to: Red cells 2,684,000, white cells 72,000.

Eight hours later this mixture was again examined. No locomotion and no evidences of phagocytosis could then be seen, but twenty-four hours later (thirty-two hours since the addition of normal blood) another examination showed that motion and phagocytosis had begun again and the cells were decidedly active.

August 22, 1907.—I examined a specimen of the patient's blood which had been taken in May, diluted (as usual) with Gower's solution and preserved in the bulb of the pipette. Even at this time, that is, after more than three months, the leucocytes were found to be still motile and phagocytic.

Possible Explanations for the Phenomena Here Described.—When the blood of this case was first examined attention was concentrated on the unusual appearances of the large phagocytic cells. What were they and whence did they arise? Were they parasites?

Were they bone-marrow giant-cells? What was the reason of their appearance?

Further study of the blood, especially with the warm stage brought out three important facts:

1. A large portion of the protoplasm of the strange cells was made up of other cells which they had ingested.

2. When free from such inclusions the majority of the phagocytic cells had essentially the characteristics of some of the "large lymphocytes" (or large, mononuclear, non-granular cells), which are seen in large numbers, for example, in malaria and in small numbers in normal blood. Every transition was found in the patient's blood between the ordinary large lymphocyte and the huge branching phagocytic cells.

3. Most important, however, was the discovery that not only the "large lymphocytes" but all the familiar leucocytes, *i. e.*, the polynuclear neutrophiles, eosinophiles, mast cells were in this case engaged in phagocytosis, though no cell type was as active as the "large lymphocyte."

This last fact suggested inevitably the inference that the presence of the enormous multitude of phagocytic cells here described was the result not of an invasion of the blood stream by foreign cells, but of changes in the serum which either (*a*) *opsonized* the red and the white cells and rendered them savory morsels for the phagocytes, or (*b*) stimulated the ordinarily feeble phagocytic powers possessed by all the leucocytes of the blood, or (*c*) produced both these results.

The presence of *auto-opsonins* or *auto-hæm-opsonins* in the patient's blood would explain all the facts of the case.

A glance at the table of differential counts on page 89 shows that the different varieties of leucocytes were present in essentially normal percentages, or, in other words, that the stimulus leading to the increase (and presumably to the phagocytic activity) of the cells was exerted on *all* the leucocytes and not on any single variety. But the presence of myelocytes and other abnormal varieties (plasma cells) suggests that we have to do in this case not merely with an aggregation of leucocytes in the peripheral blood but with a new formation in the blood-making tissues (leucæmia). Phagocytosis was performed by these abnormal varieties—myelocytes and plasma

cells—as well as by the normal varieties of leucocytes. (A plasma cell is shown in Plate V, Fig. 4, acting as a phagocyte.)

The auto-hæm-opsonic theory here advanced as an explanation of the facts in this case is further supported by the human and animal experiments which have been described.

This case and that reported by Dr. VanNuys,[3] which I believe to be practically identical with this one, are the *most marked* instances of peripheral intravascular phagocytosis; but for several years I have occasionally recognized in the peripheral blood of ordinary cases of leucæmia—both myeloid and lymphoid—phagocytosis of other blood cells performed by each of the types of leucocytes. I hope to work up and report more fully on this matter in a later paper.

No autopsy was allowed but punctures were made into the spleen, the liver, the enlarged inguinal glands and the lungs.

In the blood drawn from the spleen I found essentially the conditions described in the peripheral blood. There were also a good many giant cells from twenty to thirty microns in diameter and containing many nuclei (five to seven), sometimes peripherally arranged (Plate X, Fig. 5). To my surprise not one of the forty or fifty giant cells examined showed any inclusions or other evidence of phagocytosis. The different varieties of leucocytes already described as phagocytic in the peripheral blood were also phagocytic in the spleen.

In the blood from the lungs polynuclear leucocytes were much more numerous than in the peripheral blood and often contained cocci. The smears from liver and lymph glands showed nothing remarkable.

I wish to express my gratitude for constant assistance and advice to Dr. Stuart J. Lawson, to whom I owe the micro-photographs for this article. I have also been greatly aided by the encouragement and advice of Dr. Eugene L. Opie, Dr. Ludwig Hektoen and Dr. Mark W. Richardson; by Dr. G. G. Sears, who kindly allowed me to study the case in his wards at the Boston City Hospital, and by Dr. Richard C. Cabot, who has studied many of my specimens and verified my findings. To all of these gentlemen I wish to express my sincere thanks.

[3] VanNuys, *Boston Medical and Surgical Journal*, 1907, clvi, 390.

EXPLANATION OF PLATES.

Plate I.

Fig. 1. (*a* and *b*) × 1530. Polynuclear leucocytes with phagocytic processes starting out from the nuclei and crossing adjacent cells. Note in Fig. 1 (*a* and *b*) and in Fig. 2, the gourd-shaped enlargement at the extremities of the narrow process.

Fig. 2. (*a* and *b*) × 765. Shows a polynuclear cell with a process extending across an entire field. At the right of the field is a typical phagocytic "lymphocyte."

Fig. 3. × 765. A process from a polynuclear cell showing division into several strands.

Fig. 4. × 675. A polynuclear cell with two phagocytic processes, the longer one is attached to a red corpuscle, the other (and shorter process) splits at the end and encloses a polynuclear cell like the fingers of a hand.

Plate II.

Fig. 1. × 765. Shows a polynuclear leucocyte (distorted and torn by technique) with its process attached to and constricting a portion of the protoplasm of a lymphocyte (the arrow points to the constriction).

Fig. 2. × 1215. Shows at the top of the figure a polynuclear cell with phagocytic process attached to a lymphocyte.

Fig. 3. × 1530. Shows a process coming from a polynuclear cell in the upper left-hand portion of the figure; the process is composed of several strands. Some of them are torn, some turn and go under, but do not touch the lymphocyte which is just below the polynuclear cell.

Fig. 4. × 225. Shows the latter polynuclear cell on the extreme left and gives some idea of the length of some of the processes. The strand is broken in one place, but one of its threads remains intact and connects with the point on the right of the field where the string is again seen distinctly. The very marked increase of leucocytes can be estimated from this field.

Plate III.

Fig. 1. × 1125. A leucocyte in mitosis. With Wright's modification of Leishman's stain, the protoplasm shows the granulations of the size and color characteristic of the polynuclear leucocyte and the structure of the nucleus differs somewhat from that of any of the lymphocytes or myelocytes seen in mitosis.

Fig. 2. × 1125. One of the giant polynuclear cells, quite commonly seen in this case; when measured with a micrometer these giant polynuclear cells were found to vary very little in size. All were approximately 34 μ in diameter.

Fig. 3. (*a* and *b*) × 1125. (*a*) A polynuclear cell which has escaped after being "mauled" by a phagocyte. (*b*) A polynuclear cell showing a loosened structure and a loop in the nucleus similar to that seen on the warm stage when the nucleus was about to send out processes as the first step in phagocytosis.

Fig. 4. × 1530. (*a*) Polynuclear cell showing the amœboid processes used in locomotion (not phagocytosis). (*b*) × 1125. Pieces of protoplasm probably broken off from a polynuclear cell in the process of locomotion.

Fig. 5. × 1530 and 1125. Shows various forms taken by the polynuclear cells in the process of amœboid motion.

FIG. 6. × 1125. (*a*) A polynuclear cell with a red corpuscle included. (*b*) An "escaped" polynuclear cell whose nucleus has become circular as a result of compression exerted by a phagocyte. These "escaped" cells when watched on the warm stage showed no amœboid motion after their "escape." (*c*) Lymphocyte in mitosis.

PLATE IV.

FIG. 1. × 1530. (*a*) A polynuclear cell with the protoplasm (but not the nucleus) of a lymphocyte enclosed. The polynuclear cell shows the stretched, split appearance often seen as the result of attempts to take in an inclusion too large to be completely engulfed. (*b*) A polynuclear cell with another polynuclear partly taken in. (*c*) A phagocytic "lymphocyte" showing the nucleus still spread out so that its structure can be easily seen though the protoplasm has retracted to the usual size. Notice how tightly the nucleus appears to be stretched "around" the protoplasm. The protoplasm retracts but the nucleus seldom does.

FIG. 2. × 1125. (*a*) A polynuclear cell with a red corpuscle enclosed in an "arm" which is indicated by dark dots at the rim of the corpuscle. (*b*) × 1125 A polynuclear cell enclosing a red corpuscle, a piece of which was nipped off when the protoplasm of the polynuclear closed around it.

FIG. 3. × 1125. Shows two polynuclear cells each engaged in engulfing several red corpuscles.

FIG. 4. × 1530. (*a*) A polynuclear leucocyte with another polynuclear cell ingested and partially destroyed. (*b*) This figure shows the appearance of a polynuclear which has escaped after being partly destroyed.

FIG. 5. × 1530. (*a*) A polynuclear leucocyte showing two compartments which contain the remains of former captives. (*b*) A polynuclear leucocyte with another polynuclear cell partly ingested.

FIG. 6. (*a*) × 1125. A polynuclear leucocyte whose protoplasm has surrounded a red corpuscle. (*b*) × 1530. A polynuclear leucocyte with a red corpuscle ingested. (*c*) A lymphocyte with an inclusion.

PLATE V.

FIG. 1. × 1125. An eosinophile with a red corpuscle ingested. Below this is a polynuclear neutrophile.

FIG. 2. × 1530. An eosinophile with a polynuclear cell partially ingested. The part that is being acted upon by the enzyme of the eosinophile is stained blue while the part that is outside still retains the normal staining reaction of the polynuclear cell.

FIG. 3. × 1530. An eosinophile with two polynuclear cells ingested (the eosinophile granules do not show well in the photograph).

FIG. 4. × 1530. A cell (with similar appearance to a cell described by some observers as a plasma cell) whose so-called "vacuoles" are opaque and do not stain with Wright's modification of Leishman's stain, as can be seen by noting that some of them overlie the red corpuscle which the cell has ingested. I refer to this cell in the differential counts as a "plasma cell."

FIG. 5. × 1530. The "plasma cell" in mitosis.

FIG. 6. × 1530. A similar cell taken from a smear from the patient's spleen.

PLATE VI.

FIG. 1. × 1530. (*a*) A mast cell with a phagocytic process extended. (*b*) A mast cell with an inclusion.

FIG. 2. (*a*) × 1125. A small lymphocyte showing a "digestive vacuole" and a long protoplasmic process. (*b*) × 1530. An amœboid myelocyte.

PLATE VII.

Mechanical destruction of cells by the lymphocytes.

FIG. 1. × 1530. A lymphocyte capturing and constricting a polynuclear cell in its crescent-shaped "claw."

FIG. 2. × 1530. A lymphocyte with two polynuclear cells engulfed.

FIG. 3. × 1530. Shows a lymphocyte whose "digestive vacuole" contains a polynuclear cell which has been much compressed by the action of the phagocyte. The amount of compression and the force exerted can be estimated by comparing the size of the captive with that of the surrounding red corpuscles.

FIG. 4. × 1125. A lymphocyte with two processes extended; each process contains a piece of the cell-nucleus. Notice the blood plates assembled at the ends of each process. The lymphocyte has ingested two polynuclear leucocytes and compressed them until their nuclei are circular.

FIG. 6. × 810. Shows a single enormous lymphocyte full of inclusions of various kinds. The single loose structured nucleus of the including cell is visible near the center. An including process of protoplasm can be traced at the extreme left. This cell was found in a smear taken at a time when all the cells were especially active in phagocytosis.

PLATE VIII.

Chemical destruction of cells (perhaps by enzyme).

FIG. 1. × 1125. A lymphocyte with three processes of protoplasm extended in preparation for phagocytosis.

FIG. 2. × 1530. A lymphocyte containing two polynuclear cells in its "digestive vacuoles." (*a*) Shows the structure of the nucleus of the polynuclear cell just disappearing. (*b*) Shows a change in the structure of both the nucleus and protoplasm of the polynuclear cell.

FIG. 3. × 1530. Shows very distinctly (on the left) a polynuclear cell in the process of being destroyed.

FIG. 4. × 1530. A lymphocyte whose digestive vacuole contains but a bluish-stained mass, probably the remains of a polynuclear cell.

FIG. 5. × 1530. A lymphocyte whose digestive vacuole contains only a shell of the inclusion.

PLATE IX.

Extensive destruction of red corpuscles.

FIG. 1. × 1125. A huge multinuclear "lymphocyte" with two red corpuscles ingested and its process in the act of capturing another.

FIG. 2. (*a*) × 1125. A lymphocyte packed with captured red corpuscles and a process out at the upper end. (*b*) × 450. Shows a polynuclear cell caught at one end; below is a process of protoplasm surrounding more cells.

FIG. 3. × 1530. Cell with nine red corpuscles included.

Fig. 1.

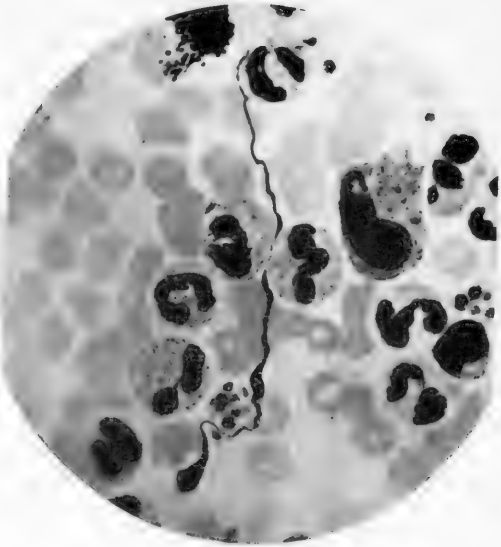


Fig. 2.

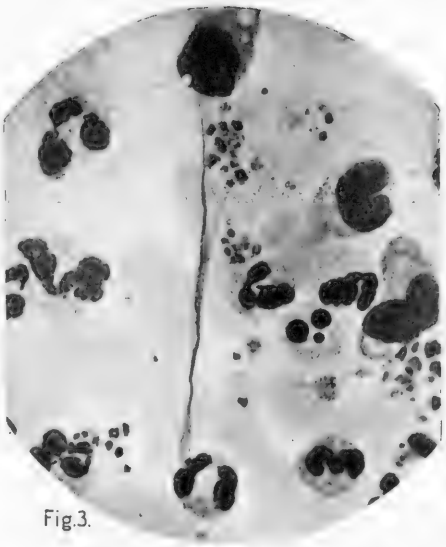


Fig. 3.

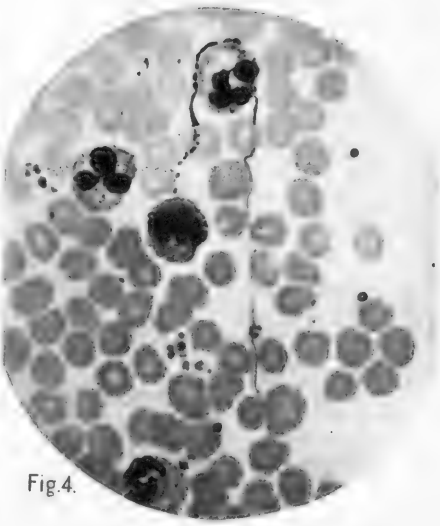
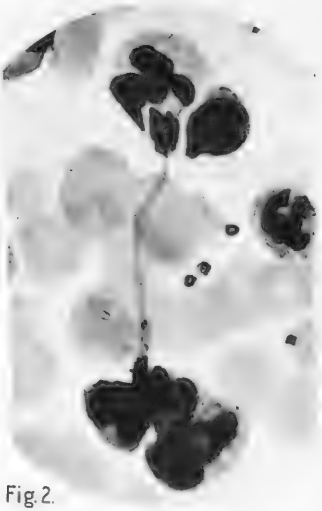


Fig. 4.

Fig. 1.



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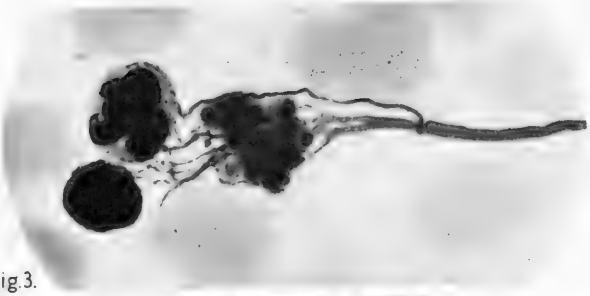


Fig. 3.

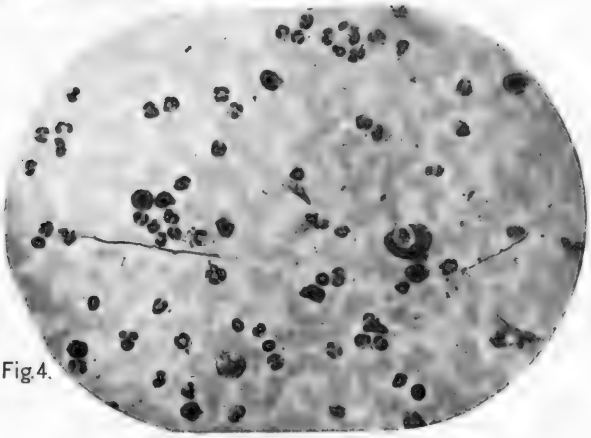


Fig. 4.

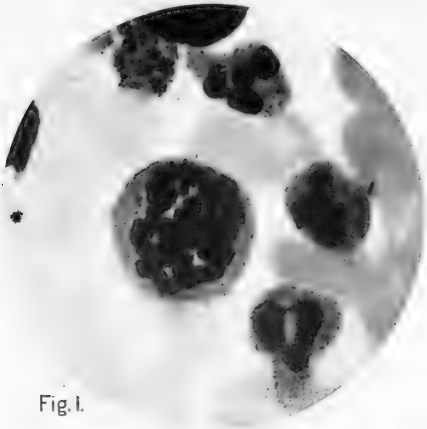


Fig. 1.

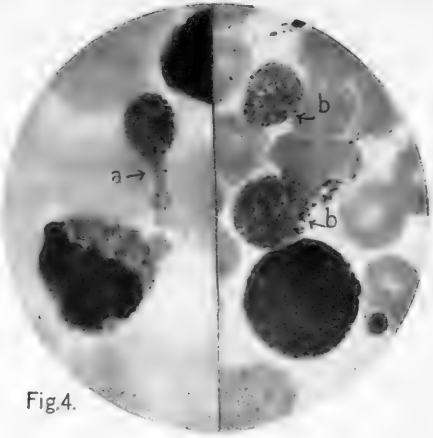


Fig. 4.

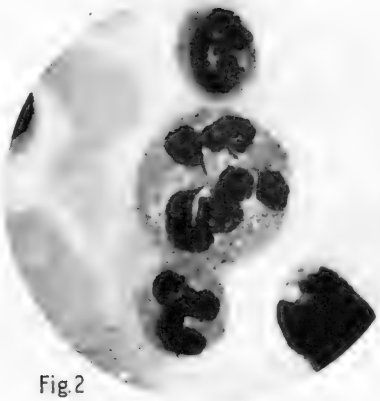


Fig. 2

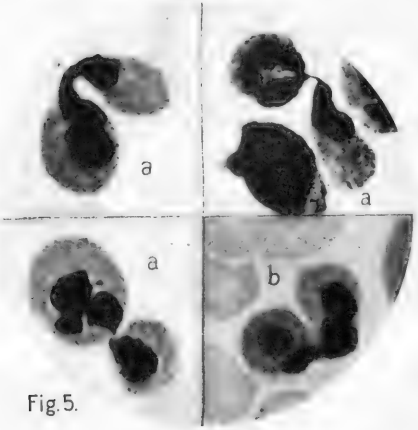
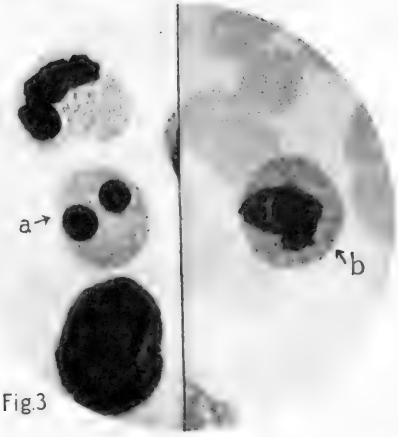


Fig. 5.



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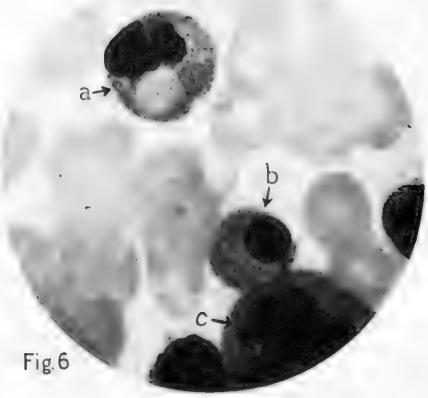

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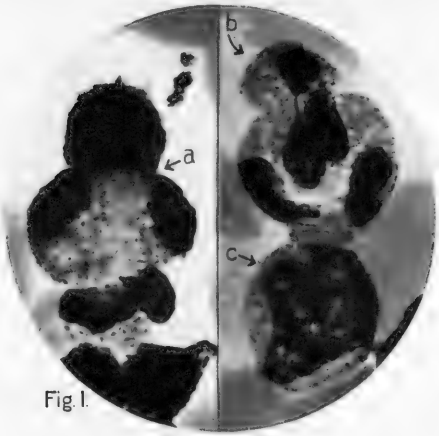

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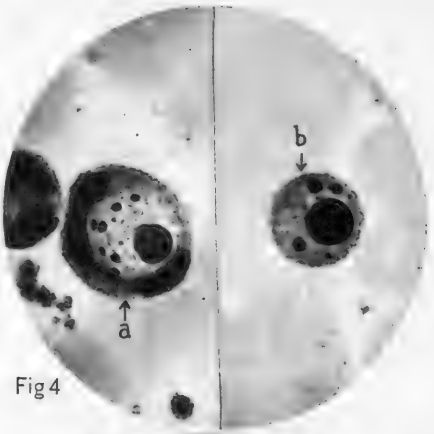

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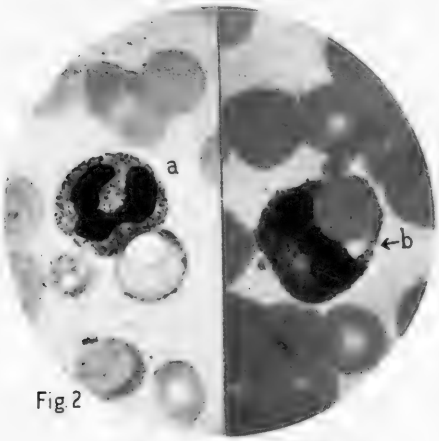

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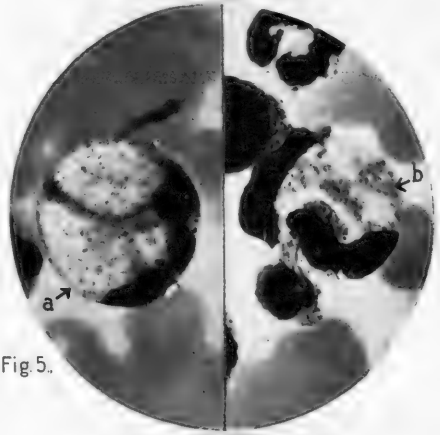

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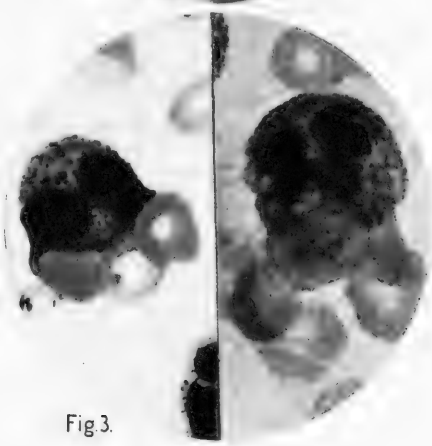

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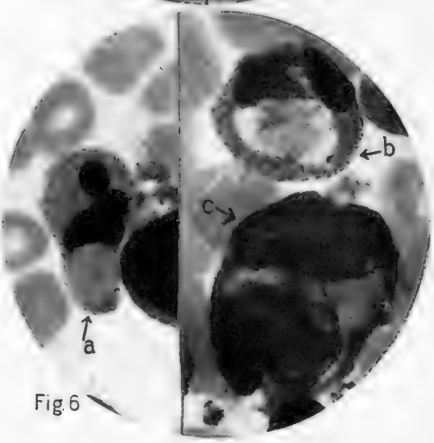

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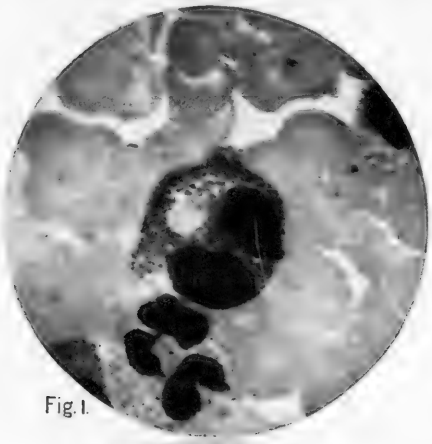

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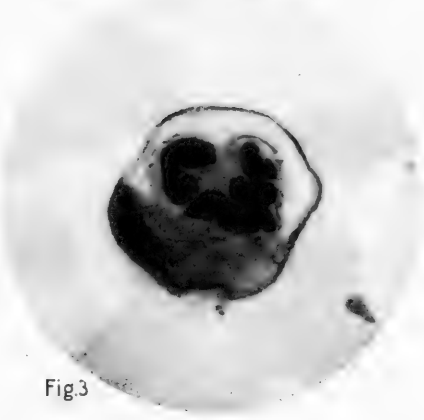

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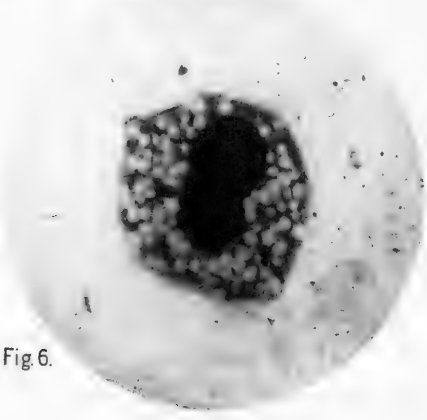

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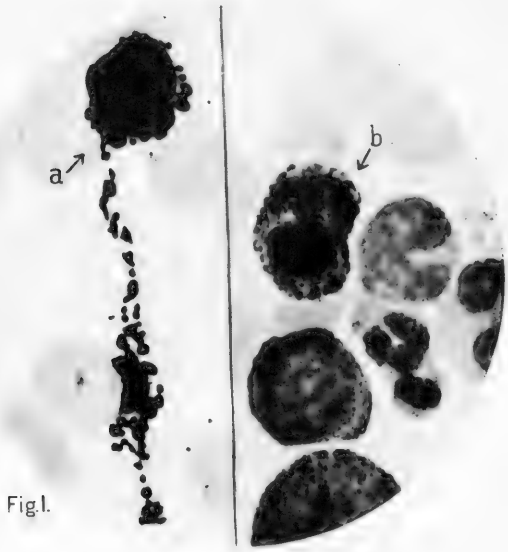

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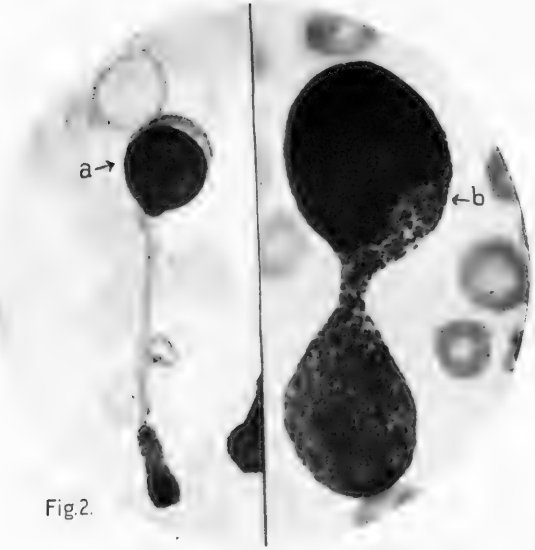

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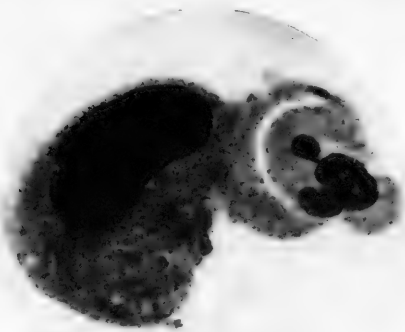

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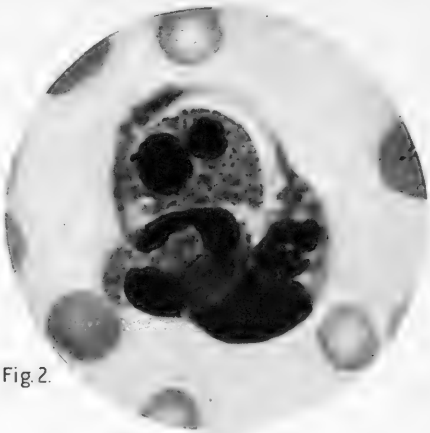

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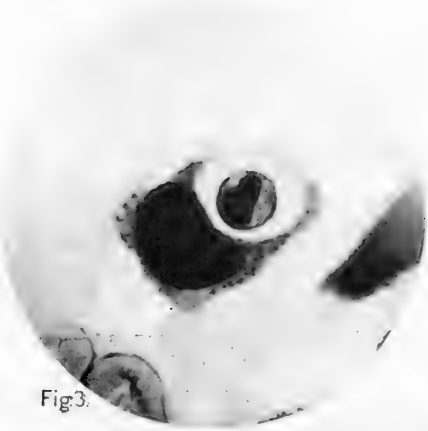

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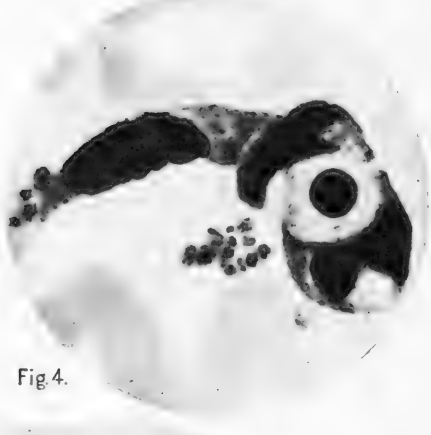

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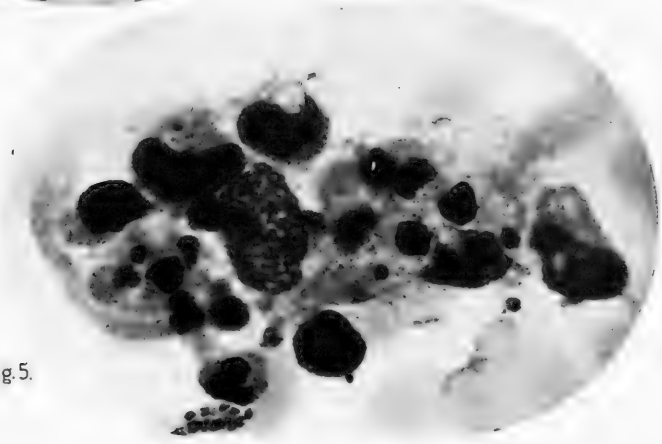

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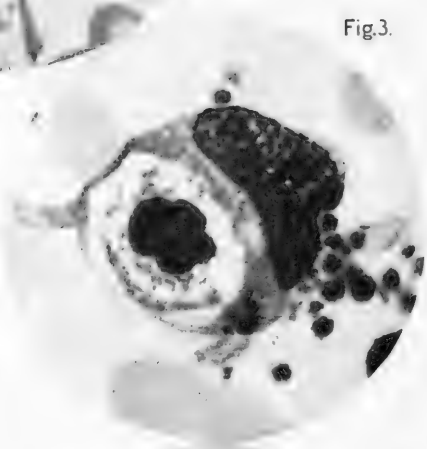

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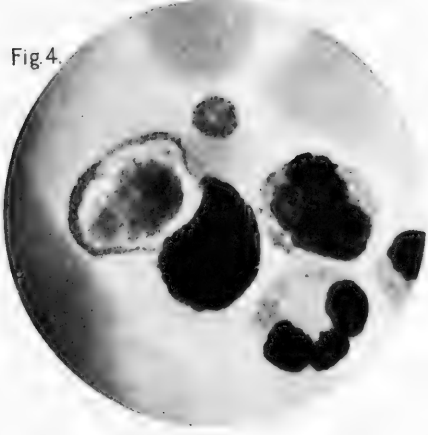

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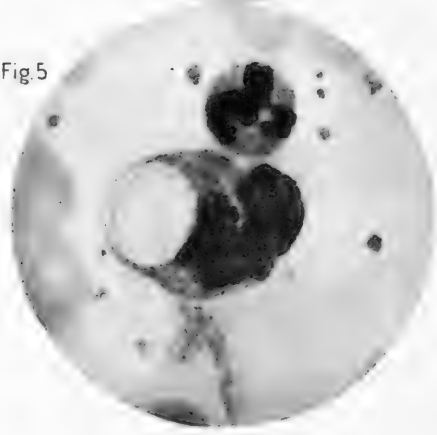

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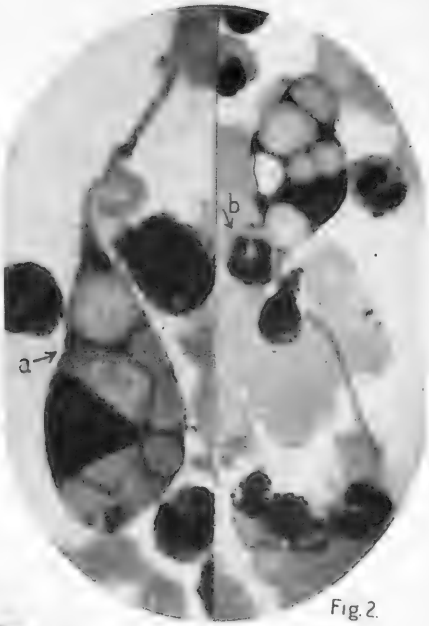

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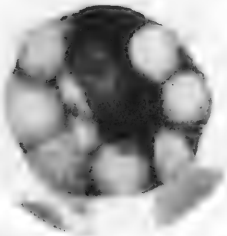

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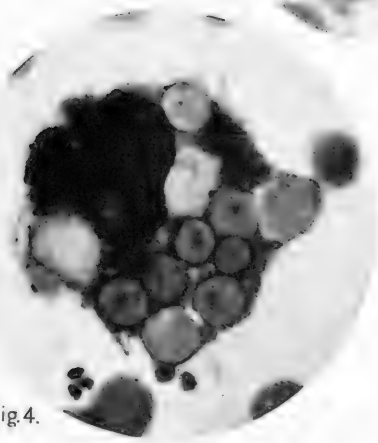

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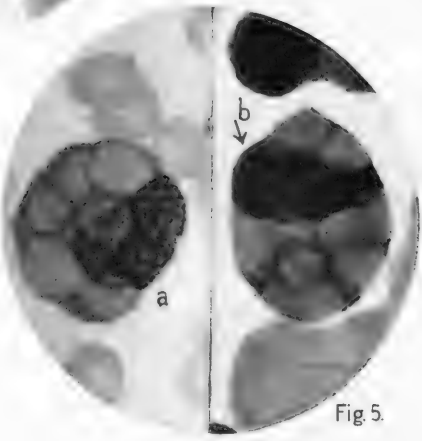

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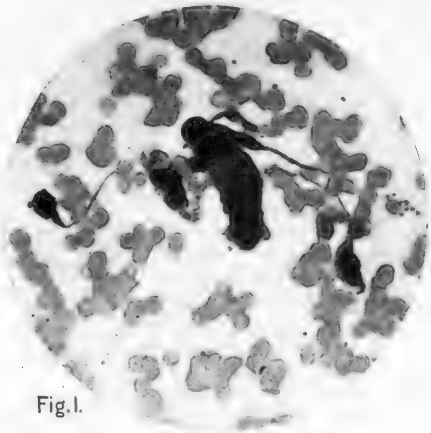

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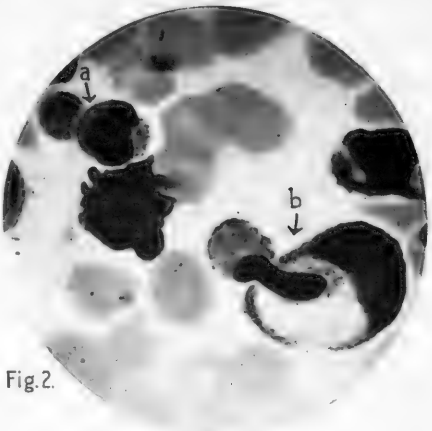

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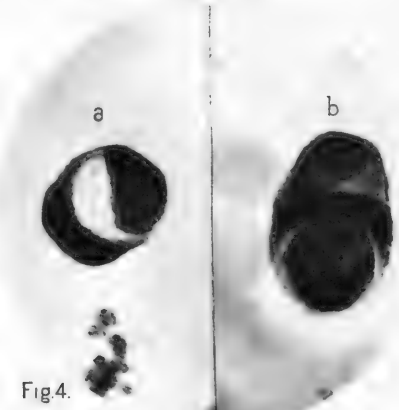

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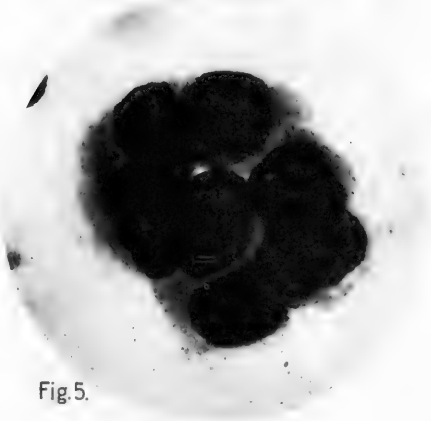

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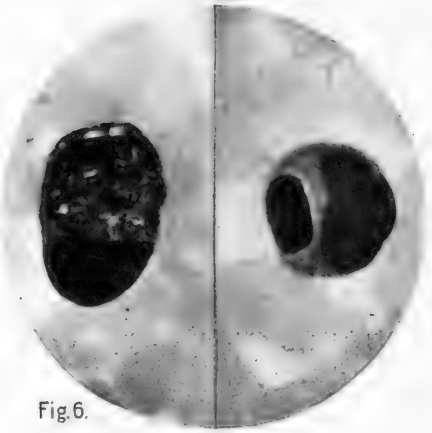


Fig. 6.

FIG. 4. × 1125. A cell with at least eleven red corpuscles included—some of them partially destroyed.

FIG. 5. (*a* and *b*) × 1530. Two cells full of inclusions. In *b* twelve red corpuscles can be counted at different levels.

PLATE X.

FIG. 1. × 450. Two lymphocytes with thin processes widened at the ends. Each of the ends contains a piece of the cell-nucleus.

FIG. 2. × 1125. (*a*) Polynuclear cell with threads from the nucleus around an inclusion. (*b*) Lymphocyte with polynuclear cell partially ingested.

FIG. 3. × 1530. A lymphocyte which has ingested another lymphocyte which itself contains inclusions.

FIG. 4. (*a* and *b*) × 1700. Two small lymphocytes with inclusions.

FIG. 5. × 1530. A giant cell taken from a smear from the patient's spleen.

FIG. 6. × 1530. Leucocytes (lymphocytes) with inclusions; from the blood of the guinea-pig after the patient's blood had been injected into the subcutaneous tissue of the animal.

TRANSPLANTATION IN MASS OF THE KIDNEYS.[1]

By ALEXIS CARREL.

(From the Rockefeller Institute for Medical Research, New York.)

I. DEFINITION.

This operation consists of extirpating from a first animal both kidneys, their vessels and the corresponding segments of the aorta and vena cava, their nerves and nervous ganglia, their ureters and the corresponding part of the bladder (Plate XI); of placing this anatomical specimen into the abdominal cavity of a second animal whose normal kidneys have been previously resected and the aorta and vena cava cut transversely (Plate XII); and of suturing the vascular segments between the ends of the aorta and vena cava, and of grafting the flap of bladder onto the bladder of the host (Plate XIII).

II. INTRODUCTION.

The purpose of the transplantation in mass of the kidneys is to reconstruct, as safely and perfectly as possible, the urinary system, when it has been suppressed by a double nephrectomy, and to study the functions of the new kidneys. It would be important to know whether kidneys extirpated from an animal and transplanted on another animal after a suspension of life of some duration, can resume efficiently their functions. No therapeutic value can be expected from the graft of kidneys unless the secretion of the new organs should be practically normal. In order to ascertain whether or not transplanted kidneys functionate in a normal way, the crucial test is certainly the grafting on one animal, having undergone previously a double nephrectomy, of both kidneys extirpated from another animal. The efficiency of the transplanted organs would be absolutely demonstrated if the host lived in good health and secreted normal urine.

[1] Received for publication October 30, 1907.

The physiology of transplanted kidneys is not known, the main purpose of the experimenters having been until now to reëstablish the circulation through the organ, and not to study its functions. When gangrene did not occur and the kidney secreted a little urine, the operation was considered a success. As the normal kidneys of the host were not resected, it was not possible to appreciate the degree of usefulness of the transplanted organ. All these experiments were made by a method that may be called simple transplantation. It consists of dissecting the kidney and cutting its vessels, extirpating and transplanting it onto some other part of the body of the same or another animal and suturing the renal vessels to other vessels, such as the jugular vein and common carotid artery, or iliac vessels, or even renal vessels.

In 1902 the first attempt of transplanting a kidney was made by Ullmann.[2] He removed a dog's kidney and transplanted it into the neck, the renal artery being united to the carotid artery and the renal vein to the external jugular vein by means of Payr's protheses. The end of the ureter was sutured to the skin and fluid flowed from the ureter, but no analysis of it seems to have been made. Three months after this communication Ullmann[3] reported having transplanted the kidney of one dog into another, and the kidney of a dog into a goat. On macroscopical examination of the kidney transplanted into the neck several necrotic areas were seen. No analysis of the urine was published.

The same year, I performed[4] on dogs several transplantations of the kidneys, at the University of Lyons. The carotid and jugular of a dog were dissected and prepared for anastomosis. The right kidney, having been extirpated, with its vessels and its ureter, was put into the cervical wound. End to end anastomoses by continuous sutures of the renal artery to the carotid, and of the renal vein to the jugular, were performed. The end of the ureter was united to a small opening of the skin a little above the sternum. After reëstablishment of the circulation a clear fluid began flowing from the ureter. Septic complications occurred in every case, and permanent results were not observed. No analysis of the urine was made. In 1902 Decastello[5] also reported experiments on the transplantation of the kidneys. He extirpated the kidney of a large dog and transplanted a kidney from another dog into its place, uniting the vessels by means of protheses. The animal lived forty hours, during which time 1,200 cubic centimeters of urine, containing a great deal of albumin and many casts, were secreted. In 1903 Carl Beck[6] of Chicago performed a transplantation of the kidney by using Murphy's method of anastomosing blood-vessels.

[2] Ullmann, *Wien. klin. Woch.*, 1902, xv, 281.
[3] Ullmann, *ibid.*, 707.
[4] Carrel, *Lyon méd.*, 1902, xcviii, 859.
[5] v. Decastello, *Wien. klin. Woch.*, 1902, xv, 317.
[6] Beck, verbal communication.

In 1905 Floresco[7] performed the transplantation of the kidney into the cervical and the inguinal regions, and in every case gangrene occurred. Then he grafted the kidney in the lumbar region; a kidney was extirpated from a dog and transplanted on another dog which had undergone the resection of one kidney, the renal vessels being united to the renal vessels of the host by continuous suturing and the ureter to the skin or to the ureter of the host. These experiments gave few facts about the functions of the kidneys. In one case a sample of fluid flowing from the ureter five days after the operation was examined and presented the characters of urea. But on the tenth day the organ was found necrosed. Floresco transplanted the kidney five times into the lumbar region. In three cases necroses occurred. In two cases the animal is reported to have lived in good health. Definitive results were not published. No analysis of urine secreted by the transplanted kidney was made.

In 1905 Guthrie and I[8] examined the functions of a transplanted kidney. The operation was performed in the Physiological Department of the University of Chicago. The kidney of a small dog was transplanted into the neck, the renal artery being sutured to the carotid artery, the renal vein to the external jugular vein and the ureter to the œsophagus. When examined three days later, the kidney was somewhat larger and its hue darker than normal. The pulsations of the renal artery were strong. The secretion of urine by the transplanted kidney was about five times more rapid than by the normal one. The intravenous injection of normal saline solution caused no change in the rate of secretion in the normal kidney, but markedly increased the rate of secretion in the transplanted organ. The constituents of the urines were similar, but the chlorides appeared more abundant in the urine from the transplanted kidney, while the organic sulphate, pigments and urea were more abundant in the urine from the normal organ.

This experiment showed that the secretion of a transplanted kidney need not be very different from the normal. However, the kidney was in an unfavorable condition. The denervation alone could not be responsible for the exaggeration of the circulation of the organ. In the neck or in the inguinal region the blood pressure is different from the pressure to which the kidney is used. Besides, its vessels, the renal vein especially, are exposed to many causes of compression and disturbance. Therefore, we gave up the idea of transplanting the kidney in a region other than the lumbar region, as Floresco did.

In 1907 Stich[9] published the results of a transplantation of the kidney in the lower abdominal region. This experiment was performed at the surgical clinic of Garre, in Breslau. The kidney was transplanted into the iliac region, the renal vessels being anastomosed, end to end, to the iliac vessels, and the end of the ureter being grafted into the bladder. The normal kidneys of the animal were not removed, and consequently, the usefulness of the transplanted organ could not be determined. The circulation remained excellent and the macroscopical and microscopical examinations showed that the organ was practically normal. The iliac region is certainly much better than the cervical region, but

[7] Floresco, *Jour. de Physiol. et de Path. generale,* 1905, vii, 27, 47.

[8] Carrel and Guthrie, *Science,* 1905, xxii, 473; *Comp. rend. de la Societé de biologie,* 1905, ii, 669.

[9] Stich, *Archiv f. klin. Chirurgie,* 1907, lxxxiii, 494.

it is probable that perfect functional results can be obtained more easily by putting the kidneys in their normal location in the lumbar region.

In 1906 I made with Guthrie[10] a few transplantations of the kidney into the lumbar region. The renal artery and vein were implanted on the wall of the aorta and vena cava by the patching method. By this method occurrence of gangrene is almost impossible, for the wall of the renal vessels is respected and the suture is made between the edges of the arterial and venous patches and the openings of the aorta and vena cava of the host. The circulation was excellent. Nevertheless there were almost always some small changes in the direction, or the situation, and in the length of the vessels, or some twisting of the vein around the artery, or a little sclerosis of the connective tissue which produced a slight obstruction of the venous circulation, chronic congestion, and after a few weeks, marked lesions of the kidney. Such an organ is not proper for the study of the functions of a transplanted kidney, for its secretion is abnormal. Its lesions and troubles of function are not due to the fact of its having been transplanted, but merely of its having been transplanted with an imperfect technique.

For studying the functions of transplanted kidneys, the transplantation in mass appeared to be the ideal method. It permits an almost perfect reconstruction of the urinary apparatus, and prevents the occurrence of gangrene and, in a large measure, of secondary lesions of the parenchyma, since the renal vessels themselves are not disturbed and the ureters not severed. It may preserve a part of the nervous system of the organs, and places the organs in a condition as near as possible to normal.

The first transplantation in mass of the kidneys was performed in 1906 by Guthrie[11] and myself in the Physiological Laboratory of the University of Chicago.

Both kidneys and the upper part of the ureters of a dog, together with their vessels, nerves, nervous ganglia, the surrounding connective tissue, the suprarenal glands, the peritoneum and the corresponding segments of the aorta and vena cava were removed. The mass was then placed in a vessel of isotonic sodium chloride solution. The aorta and vena cava of a bitch were cut a little

[10] Carrel and Guthrie, *Jour. of Amer. Med. Assoc.*, 1906, xlvii, 1648.

[11] Carrel and Guthrie, *Science*, 1906, xxiii, 394; *Comp. rend. de la Societé de biologie*, 1906, i, 465.

above the mouth of the ovarian vessels. The kidneys of the first animal were then removed from the salt solution and put into the abdominal cavity of the second, and the segments of the aorta and vena cava were interposed, by biterminal transplantation, between the cut ends of the aorta and vena cava. The circulation was reestablished, after having been interrupted for one hour and a half. The kidneys immediately became red and turgid, as after a simple transplantation, but about half an hour later the state of the circulation became normal. Clear urine flowed abundantly from the transplanted ureters, which were united to the normal ones.

Both normal kidneys were then removed. Two hours after the operation the animal walked about her cage. In the afternoon she drank and urinated copiously. The following day and subsequently, up to the eighth day, her diet consisted largely of meat, which she took hungrily. In general her condition was normal. During this period the urine remained clear, showing no evidence of blood. The total amount appeared to be somewhat increased. In the seventh and eighth days several samples were collected and analyzed, the results of which showed a slight variation in composition, but entirely within normal limits. The only abnormal constituent detected was coagulable proteid, the largest amount present in any of the samples being less than 0.25 per cent.

On the ninth day she vomited. A diagnosis of stenosis of the bowels by adhesions was made. On the tenth day the urine was analyzed and its composition was practically the same as before. As the animal was vomiting almost continuously, she was etherized and a laparotomy performed. We found localized peritonitis on the right side of the abdomen, with kinking of several loops of intestines, the mass being very strongly bound down by adhesions. The circulation of both kidneys was found to be perfect. There was an enormous hydronephrosis on one side.

Afterwards a few other transplantations on dogs and cats were performed without obtaining better results. The animals died rapidly from intestinal, peritoneal or ureteral complications.

In 1907 at the Rockefeller Institute, I modified and improved the technique in order to suppress, as much as possible, the occurrence of complications. A few operations were made on dogs and on cats and it appeared soon that, in spite of the difficulties due to the smallness of the vessels, cats are better adapted from an anatomical standpoint for this operation than dogs. When the details of the operation had been worked out fourteen experiments were performed from February to October, 1907. Progressively the technique was improved and the cause of the complications which occurred ascertained, and as far as possible suppressed. The actual technique, however, must not be considered as a definitive one. It will be modified in many respects, as greater experience in performing it is obtained.

III. TECHNIQUE.

The transplantation in mass of the kidneys can be described as consisting of four stages: (*a*) preparation of the kidneys; (*b*) perfusion and extirpation of the kidneys; (*c*) preparation of the host; and (*d*) graft of the kidneys into the abdomen of the host.

It is evident that all resources of modern surgery must be used to prevent infection and shock after such an operation, which necessitates a large transverse incision of the abdominal wall, the evisceration of the intestines and the spleen, a double nephrectomy, the stopping of the aortic and caval circulations, the section of the aorta and vena cava, and the opening of the bladder. The animal is handled and operated on with the same rigid asepsis and care used for a human patient in a well-equipped hospital.

The order of the succession of the different stages of the operation is variable. We shall describe the simplest form, when the four stages are united in two: preparation and extirpation of the kidneys; preparation of the host and graft of the kidneys.

A. *Preparation and Extirpation of the Kidneys.*

A first animal is etherized. After shaving and sterilization of the abdominal and lumbar regions, the antero-lateral wall is cut transversely at the level of the umbilicus to about one half or three quarters of the circumference of the animal. The intestines are eviscerated and put on the left side of the body. Thus both kidneys and the lumbar region are largely exposed.

1. *Dissection of the Kidneys.*—The peritoneum is cut all around the kidneys in a rectangular shape, the short sides being along the external edge of the kidneys, the long sides uniting the ends of the organs and being perpendicular to the vena cava. Care must be taken not to wound the ureters and the vena cava. Through the peritoneum it is easy to see the point of implantation of the renal veins. At about one centimeter above the mouth of the right renal vein the vena cava is dissected and isolated from the lower end of the right suprarenal gland. On the left side of the vessel, just above the left renal vein, is often the mouth of the left suprarenal vein. This vein is ligated and cut, if the left suprarenal gland is not to be transplanted with the kidneys. Afterwards, the vena

cava is dissected on a point located at two or three centimeters below the mouth of the left renal vein. The left genital vein is ligated and cut. Then the aorta is dissected at two points located one and one half centimeters above and below the renal arteries. The kidneys are detached from the lumbar region by cutting the loose connective tissue which is interposed between them and the muscular plane. The posterior part of the aorta and vena cava is dissected. The posterior collateral branches of these vessels are ligated and cut.

The ureters are dissected from the lower edge of the peritoneal flap to the bladder. Their vessels must be carefully respected. Afterwards the part of the bladder on which the ureters are implanted is dissected. The musculo-peritoneal layer of the bladder is cut by a circular incision located at about one centimeter from the mouth of the ureters. Then the mucous membrane is easily seen and cut, in such a way, with the scissors, that the mucous flap is larger than the muscular one. The ureters and the flap of bladder are still fixed by the rectum and the uterus. The uterus is cut, and the meso-colon severed as far as the cæcum. The small intestine is cut and through the solution of continuity the ureters and the fragment of bladder are removed. Great care is taken to prevent infection by the section of the intestines.

If the left suprarenal must be transplanted, its upper vessels are ligated and cut. The last connective tissue adhesions are severed. The anatomical specimen is yet adherent to the animal by the aorta and vena cava.

2. *Stopping of the Circulation, Perfusion and Extirpation of the Kidneys.*—The aorta is clamped below the diaphragm. A glass cannula is introduced through the lower part of the abdominal aorta and connected with an irrigator containing Locke's solution at the temperature of the laboratory, which was from 26° to 37° C. The vena cava is cut one centimeter above and two or three centimeters below the renal veins. Then the aorta is washed and the kidneys are perfused with Locke's solution. In the first experiments they were washed until their surface became yellowish white and the fluid flowing from the vena cava was perfectly clear. In the last experiments the perfusion was much less complete. It was stopped

as soon as the blood in the vena cava appeared to be greatly diluted, and when there was still a good deal of blood in the kidneys.

Next the aorta is cut one centimeter or one and a half centimeters above and about two centimeters below the renal arteries. The anatomical specimen is removed (Plate XI) and put into a jar of Locke's solution at the temperature of the laboratory.

B. *Preparation of the Host, Double Nephrectomy and Graft of the New Kidneys.*

A second animal of the same size or a little larger than the first one is etherized. The abdomen and the lumbar region are clipped, shaved and carefully sterilized. The abdominal wall is transversely cut at the level of the umbilicus from one lumbar region to the other. The intestine and the spleen are eviscerated and wrapped in a towel or in a greased silk pad, and put outside on the left side of the body. They are protected there by a small wool blanket, in order to prevent cold and the consequent shock. The aortico-renal region is then largely exposed. When the bladder is too distended by urine, it is put outside of the abdominal cavity and covered with greased pads.

1. *Extirpation of the Kidneys* (Plate XII).—The lumbar peritoneum is cut longitudinally on the middle line, its edges dissected at the level of the renal veins. The pedicles of the kidneys are dissected and a ligature is put on each structure, artery, vein and ureter. Afterwards through the longitudinal peritoneal incision, both kidneys are extirpated in such a manner that the constitution of the renal region is not disturbed at all. Both suprarenal glands are respected, their lower vessels being sometimes ligated. The left genital vein is ligated.

2. *Preparation of the Vessels.*—The vena cava and the aorta are prepared for anastomosis. The vena cava is dissected at the point of implantation of the renal and the left suprarenal veins. One or two lumbar collaterals, and sometimes the right genital vein are ligated. The temporary hæmostasis is secured by *serre fines* especially modified for this purpose. A *serre fine* is put on the vena cava four centimeters below the renal veins, and another one as high as possible, about two centimeters above the right renal

vein. Then the region on which the renal veins are implanted is resected (Plate XII). Through the cut ends of the vessels a curved glass cannula is introduced, and the blood is washed out with Locke's solution. A little vaseline is put into the lumen and on the external part of the vein. The aorta is generally dissected just below the renal arteries; the first two lumbar collaterals are ligated, a *serre fine* is put on the wall of the vessel just below the renal arteries, and another three centimeters lower. It is better, but involves greater risk, to dissect the aorta above the renal arteries, in order to place the *serre fine* higher and to make the anastomosis a few millimeters below the implantation of these vessels. The relations of the thoracic duct and of the aorta are so close at that point that the duct is exposed to injury while the aorta is being dissected. It is much safer to put the higher *serre fine* below the renal arteries. Generally the aorta is cut two centimeters below the renals, or sometimes a very short segment, with the implantation of the first two lumbar collaterals, is resected. The aorta being very elastic there is immediately a gap of two or three centimeters between the cut ends of the vessel (Plate XII). The external sheath is resected for a short distance. The blood is washed out from the lumen of the vessel with Locke's solution. The lumbar region is carefully washed with Locke's solution and dried with gauze in order to remove all traces of blood. Then the vessels are washed again, and covered with a thin layer of vaseline. Vaseline is also put on the surounding anatomical structures.

3. *Transplantation of the Kidneys.*—The anatomical specimen is removed from its jar and put into the abdominal cavity. Each kidney is introduced through the incision of the lumbar peritoneum into the corresponding renal region. The new kidneys, being about the same size as the extirpated ones, fill exactly their place. The aortic and caval segments are interposed between the cut ends of the aorta and vena cava. The ends of the vascular segments are washed, if necessary, and greased with vaseline. Afterwards the small region of the vessels is circumscribed with black silk towels, for it is necessary to protect the threads from all contact with blood or with plasmase of the tissues.

4. *Anastomoses of the Vessels and Reëstablishment of the Circu-*

lation.—The ends of the aorta are united to the ends of the aortic segment by continuous suturing with straight needles, No. 16, and silk thread boiled in vaseline. Care is taken to express the vaseline from the lumen of the vessel before completing the suture. Afterwards the anastomoses of the ends of the venous segments to the ends of the vena cava are made. The wall of the vein is very thin, and it is necessary to apply intima to intima while suturing, that is, to make a slight eversion of the wall. It is the safest way to prevent inclusion of the external sheath into the line of sutures.

After completion of the anastomoses dry gauze is applied on the anastomoses and the *serre fines* are removed first from the vena cava and secondly from the aorta. For about two minutes a slight compression is made in the region of the anastomosis. Afterwards the gauze is removed and, if there is still a little leakage, one or two complementary stitches are added. It is necessary that no blood at all flow from the line of sutures. The hæmostasis must be absolute.

As soon as the circulation is reëstablished through the aorta, the kidneys begin to be injected with blood, and generally after a few minutes, they assume their normal appearance. The duration of the interruption of the circulation is about one hour. Gauze with Locke's solution is put on the vessels.

5. *Graft of the Flap of Bladder on the Bladder of the Host, and End of the Operation.*—The ureters are extended on the free surface of the peritoneum along the right side of the rectum and along the right uterine horn. The bladder is isolated by pads from the peritoneal cavity. On the middle line of its posterior face a longitudinal incision is made. The flap of bladder is grafted into that opening by muco-mucous, and musculo-muscular continuous sutures. Then the bladder is put back into the abdominal cavity.

The arterial and venous anastomoses are examined again, and if there is slight leakage, it is stopped. If a little blood flows from some small vessels of the connective tissue a ligature is applied, even if the hæmorrhage is exceedingly small. The vaseline and the blood which may be present in the connective tissue around the vessels are washed out with warm Locke's solution.

The lumbar region is closed by suture of the longitudinal peritoneal incision. Thus both kidneys are fixed in their normal location. The intestines and the spleen are put back into the peritoneal cavity. If the intestines are a little cold about one hundred grams of warm Locke's solution are injected into the peritoneal cavity.

The peritoneum and the abdominal muscles are closed by two planes of continuous silk sutures. The skin is sutured with catgut. The dressing consists of talcum powder, gauze, cotton, bandage and a linen shirt.

After the operation the animal is put for two hours into a cage heated to about 30° C. and then into an ordinary cage with screen floor or saw dust. No special care is taken. The animal is given meat and milk one day after the operation. The dressing is removed after about six days. When the urine is to be examined, the animal is put into a metabolism cage with fine screen floor. Only incomplete analyses of urine have been made by using the ordinary clinical methods. In spite of their lack of accuracy they are sufficient to prove that the kidneys functionate. It is not intended, in this article, to analyse minutely the changes in the secretion of transplanted kidney, but merely to show that after transplantation they can resume their function efficiently. This is demonstrated as much by the general condition of the animal as by the physical and chemical characters of the urine secreted by the new kidneys.

IV. EXPERIMENTS.

Fourteen experiments have been performed.[12] Two animals whose ureteral anastomoses were defective died soon after the operation. No excretion of urine was observed from the bladder, because it flowed into the retroperitoneal spaces. Three other animals, operated on under unfavorable condition died almost immediately of shock. These five experiments will not be reported. Thus nine cases only will be described.

Experiment 1. February 25, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. Etherization and semi-

[12] Several of these operations were made with the aid of Mr. R. D. McClure of Johns Hopkins University, whom I wish to thank for his assistance.

circular transversal laparotomy. Evisceration. Dissection and isolation from the surrounding structures of both kidneys, the left suprarenal gland, their vessels and the corresponding segments of the aorta and vena cava, their nervous system, and the upper part of the ureters, which are cut about 6 centimeters below the hilus. Opening of the lower part of the abdominal aorta. Animal killed by hæmorrhage and opening of the diaphragm. Section of the vena cava 1.5 centimeters above and 3 centimeters below the mouth of the renal veins. Introduction of a cannula through the abdominal aorta. Washing of the aorta and perfusion of the kidneys with very hot (by accident) Locke's solution, until their color became yellowish-white and the fluid flowed perfectly clear from the vena cava. Then section of the aorta about 1.5 centimeters above and 1.5 centimeters below the mouth of the renal arteries. Anatomical specimen removed and put into Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Male, young, black cat, good health. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestine on the left side of the body. Longitudinal incision of the lumbar peritoneum in the middle line, through which both kidneys are dissected and extirpated. Dissection of the vena cava at the level of the renal veins. Ligation of two posterior collateral branches. Dissection of the aorta, which is isolated from the thoracic duct. Ligation of both first lumbar collateral branches. Temporary hæmostasis of a segment of aorta by two *serre fines* put on the vessel just above and 4 centimeters below the renal arteries. Section of the aorta a little above the mouth of the first lumbar branches. Resection of a segment of aorta about 5 millimeters long. Temporary hæmostasis of the vena cava by two *serre fines* placed just above the right renal vein and the right spermatic vein. Resection of the point of implantation of the renal veins. Washing of the ends of the vessels with Locke's solution and greasing with vaseline.

The anatomical specimen is then removed from the jar and put into the abdominal cavity, each kidney being placed under the lumbar peritoneum in its normal location. Interposition of the aortic and caval segments between the cut ends of the aorta and vena cava. Anastomosis of the vessels by suture. No expression of the vaseline from the vessels before completion of the suture. The *serre fines* are taken out and the blood flows through the aorta and vena cava. Slight leakage from the venous anastomoses, which stops by compression. No leakage of the arterial anastomoses.

Very slow reëstablishment of the circulation through the kidneys. Nevertheless, after a few minutes, the right kidney assumes a rosy color, without blue or white spots, while the left kidney remains paler. The lower end of the left kidney is white. Small incision made at this point. Slight hæmorrhage of red blood mixed with vaseline. Progressive improvement of the circulation of the left kidney. After thirty minutes its color is rosy. Circulation almost normal.

Invagination of the end of the transplanted ureters into the upper end of the ureters of the host, and fixation of the invagination by three stitches. Closing of the lumbar peritoneal incision. Reintegration of the intestines into the abdominal cavity. Closing of the abdominal wall by three planes of catgut sutures. Gauze and cotton dressing, linen shirt.

February 25, 4 p. m. Cat in good condition. A little depressed.

February 26. Cat in good condition, walks about its cage, drinks water, but does not eat. Femoral pulse normal. A little urine.

February 27. Cat in good condition, drinks water and milk, walks, does not eat meat.—9 a. m. Urinates abundantly.—4 p. m. Urinates again. Yellowish urine, acid reaction, albumin present.

February 28. Good condition. Normal femoral pulse. Animal drinks milk, urinates abundantly, walks about the room. Clear, pale, yellow, acid urine. Albumin.

March 1. Animal in very good condition. Urinates abundantly. Clear, yellowish urine. Less albumin.

March 2. Same condition.

March 3. Animal ill, does not drink milk. Coughs, discharge through the nose. Urinates.

March 4. Animal very ill, coughs, diarrhœa, discharge through the nose. Urinates abundantly.

March 5. Same condition. Animal emaciated. Abundant urine. Drinks water.

March 6. Animal in better condition. Diminution of the discharge through the nose and of the diarrhœa. Drinks milk. Walks about its cage and the room.

March 7. Much better condition. No more discharge. A little diarrhœa. Less abundant urine. Animal hungry and drinks milk, eats fish and raw meat.

March 8. Animal drinks milk and eats raw meat. Urinates. Walks about the room.

March 9. Animal ill, refuses to eat. Urinates as usual, stays in cage.

March 10. Animal very ill. Not able to jump from its cage to the floor. No vomiting, no dyspnœa, no shaking. Urinates. Pressure on the abdominal wall is painful. In one point dressing is wet.—11 a. m. Etherization. Section of the dressing. Several loops of intestines in the gauze, the abdominal wound being almost completely disunited: premature resorption of the catgut. The intestinal loops are partially protected by the omentum which is adherent to the lower edge of the abdominal wound. Comparatively little inflammation of the peritoneum. However, four intestinal loops are adherent to the gauze dressing and very much inflamed. Adhesions are detached and intestine washed. Direct examination of the kidneys: normal peritoneal covering, normal location, marked enlargement, almost normal consistency. They look like normal hydronephrotic kidneys. Washing of the abdominal cavity with warm water. Closing of the abdominal wound by silk suture. Shock. Afterwards respiration and pulse become progressively almost normal—4 p. m. Animal able to walk.

March 11. Died in the morning.

Autopsy.—General peritonitis.

Macroscopical Examination.—Both kidneys in normal location, covered with transparent peritoneum. Normal hue, increased size. Normal consistency.

The specimen is not dissected, but preserved as demonstration specimen. Incision on the external edge of the left kidney. Urine under pressure flows from the incision. The surface of section is a little congested. No infarction. Dilatation of the pelvis and calices. It is a typical hydronephrotic kidney. Right kidney not opened, but a knife is introduced deeply into the renal substance, and urine escapes along the blade. Same hydronephrotic condition on the right side. Opening of the vena cava. Anastomoses perfect. No dissection of the aorta. No dissection of the ureters. Specimen preserved in formalin.

Microscopical Examination.—Fragment of the left kidney in Zenker's fluid stained in hematoxylin and eosin. Glomeruli well preserved. In some of them slight exudate between capillaries and the capsule. Secreting tubules slightly dilated. Epithelium in good condition; brush border well defined, nuclei normal. Excretory tubules very much dilated. Around some of them slight small-cell infiltration. A little dilatation of the blood-vessels. Appearance of an ordinary hydronephrotic kidney.

Experiment 2.—March 14, 1907.

Extirpation of the Kidneys.—Small pregnant cat etherized and killed by aortic hæmorrhage. Dissection and isolation in one mass of the kidneys, their vessels, their nerves and the upper part of the ureters. Left suprarenal gland not extirpated. By an intra-aortic injection of Locke's solution both kidneys are washed out very thoroughly, until all blood is expelled and their color becomes pure yellowish-white. Anatomical specimen extirpated and put in Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Large, gray, young, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration. Extirpation of both normal kidneys. Dissection of the aorta and vena cava. Ligature of the lower suprarenal vein and section. Ligation of the first lumbar collateral branches of the aorta. Temporary hæmostasis of the aorta by tapes fixed by *serre fines*, and of the vena cava by *serre fines* put directly on the wall of the vessel. Resection of a narrow segment of aorta in which the first lumbar arteries are implanted. Resection of a long segment of vena cava where are implanted the inferior suprarenal vein and both renal veins.

The anatomical specimen is then put into the abdominal cavity, the kidneys being in their normal location and the vascular segments interposed between the cut ends of the aorta and vena cava. Arterial and venous anastomoses by the ordinary method. Reëstablishment of the circulation through the vena cava. Leakage at one point of the lower anastomosis; hæmorrhage controlled by one supplementary stitch.

Reëstablishment of the circulation through the aorta. Hæmorrhage of the upper anastomosis, which is controlled by two additional stitches. Slow reëstablishment of the circulation through the kidneys. Appearance of both kidneys normal twenty minutes after the reëstablishment of the arterial circulation.

Anastomoses of the ureters to the upper portion of the ureters of the host by invagination. Suture of the lumbar peritoneum. Reintegration of the intestines in the abdominal cavity. Closing of the abdominal wound by two planes of silk suture and one plane of catgut suture. Gauze and cotton dressing. Shirt.

March 15. Animal in good condition, drinks milk and eats raw meat. Large amount of clear urine. In the afternoon several fits of abdominal pain.

March 16, 9 a. m. Animal less well, refuses to eat, drinks a great deal of water and milk. Since yesterday at 6 p. m. 130 c.c. of urine. Urinates again at 10.30 a. m. yellow, clear, acid urine. A great many spermatozoa, no red blood corpuscles, a little albumin. In the afternoon, fits of abdominal pain, vomited once, no feces. In the evening animal very ill.

March 17. Animal died in the morning.

Autopsy.—A little reddish fluid in the peritoneal cavity. A loop of small intestine is found dilated and of dark color: volvulus. Both kidneys are normal in size, color and consistency. Arterial and venous anastomoses perfect.

Experiment 3.—June 13, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. 10.38 a. m. Etherization. Kidneys exposed and dissected the same as before. Dissection of the aorta and vena cava at two points about 1.5 centimeters above and 2 centimeters below the mouth of the renal vessels. Section of the ureters about 6 centimeters below the hilus. The animal is killed by chloroform at 11.15 a. m. Section of the vena cava. Perfusion of both kidneys through the aorta with Locke's solution at the temperature of the laboratory (30° C.). Complete washing. The fluid from the vena cava is clear, and the kidneys are yellowish-white. Section of the aorta 1 centimeter above and 2 centimeters below the renal arteries. Then the kidneys, the left suprarenal gland, the upper part of the ureters with their vessels and their nerves are removed and put in Locke's solution.

Transplantation of the Kidneys.—Large, yellow, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen and extirpation of both kidneys. Ligature of the lower suprarenal vein, of two venous collaterals of the vena cava, and the first pair of aortic lumbar collaterals. Dissection of the aorta and vena cava and temporary hæmostasis with *serre fines*. Transverse section of both vessels. The anatomical specimen is then put in the abdominal cavity, the new kidneys being in the same location as the kidneys of the host. Anastomosis of the aortic and caval segments. Re-establishment of the venous and arterial circulation at 12.20 p. m. No leakage of the anastomoses. Immediate reëstablishment of the circulation through both kidneys. No blue or white spots. Normal color and consistency. No apparent vasodilation.

Anastomosis of the ureters by invagination. Suture of the lumbar peritoneum. The intestines and the spleen are replaced into the peritoneal cavity. A few cubic centimeters of Locke's solution are let into the peritoneum. Closing of the abdominal wound by two planes of silk sutures and one plane of catgut. Gauze and cotton dressing. Shirt. Animal is put in a metabolism cage.

June 13, 4 p. m. Animal in satisfactory condition, lays down, does not walk, drinks water.

June 14, 9 a. m. Animal in good condition, walks about his cage, and drinks milk. In the jar 250 c.c. of a mixture of urine, vomitus and a little milk.—9.30 a. m. Animal urinates, clear, slightly rosy urine, quantity 42 c.c., reaction acid, density 1.021, a great many blood corpuscles, a few spermatozoa, no casts.

June 15, 9 a. m. Animal in good condition, drinks milk. Urine 110 c.c. mixed with feces.—3.45 p. m. Clear, rosy urine, with many blood corpuscles.—4.35 p. m. Animal urinates again. Urine 38 c.c., clear, slightly rosy, acid reaction, density 1.018, urea 2.5 per 100 c.c., albumin less than 0.50 g. per 1000 c.c. Many red blood corpuscles, a few spermatozoa, no casts.

June 16, 9 a. m. Vomits a little milk. In the jar 205 c.c. urine and milk.

June 17. Animal in good condition, walks about the room, drinks water, but refuses milk and meat. Does not vomit. Urine mixed with feces.

June 18. Urine 125 c.c., yellowish, acid, density 1.016, urea 2.8 per 100 c.c., traces of albumin. Animal refuses to eat.

June 19. Animal grows emaciated, drinks a great deal of water. Urine 164 c.c., pale yellowish, clear, density 1.013, very little albumin, urea 2.3 per 100 c.c. urinates again 35 c.c. at 1 p. m.

June 20. During the night the animal has escaped from its cage, and urinated on the floor. He is emaciated and refuses to eat.—10.30 a. m. Urinates again, urine 35 c.c., clear, density 1.013 with traces of albumin.

June 21, 7.30 a. m. Urine 115 c.c., alkaline reaction. Density 1.012, urea 2.6 per 100 c.c., no albumin.

June 22, 7.15 a. m. Urine 167 c.c., density 1.017, no albumin. Animal is much emaciated, and does not eat.

June 23. Urine 110 g. Animal is very weak, nevertheless he can walk about the room. The dressing is removed. Wound completely healed. Animal very much emaciated, kidneys a little enlarged. Does not vomit.—11 a. m. Transfusion of blood by Crile's method through an anastomosis of the carotid artery of another cat to the external jugular vein. Blood is transfused until the second animal dies from hæmorrhage.

The animal is now in better condition. Pulse much stronger. The mucous membrane of the mouth and the skin of the feet are red. A few minutes after the operation clonic convulsions of the jaws and the limbs. Afterwards tonic convulsions. Tetanus-like appearance.—5 p. m. Died.

Autopsy.—Macroscopical Examination.

Opening of the abdomen a few minutes after death. Excellent healing of the abdominal wound. No adhesions of the intestines. In the gastric region long and narrow strand compressing the duodenum just below the pylorus, without, however, producing complete occlusion. Both kidneys are increased in size, their color, their location and their consistency are almost normal. Section of the right kidney: dilatation of the pelvis and calices, cortex and medulla apparently normal, although a little congested. Capsule normal. Upper part of the ureter greatly dilated. Incision of the right ureter: stenosis of the invaginated part; below this point the ureter normal. The left ureter too is very much dilated and sinuous above the point of anastomosis.

Bladder filled with 35 c.c. yellow, clear urine, density 1020, urea 2.9, no albumin.

Vessels of the kidneys normal. Aorta and vena cava dissected and incised. No thrombosis or stenosis, perfect anastomoses. No sclerosis of the perivascular connective tissue.

Microscopical Examination.—Piece of the right kidney fixed in Zenker's fluid, stained in hematoxylin and eosin. Glomeruli normal, secretory tubules slightly dilated, epithelial cells in good condition. Excretory tubules somewhat dilated. Very few light casts. No interstitial infiltration. At a few points a few mononuclear leucocytes around the tubules.

Experiment 4.—June 19, 1907.

Preparation of the Host.—Young, white and black male cat in very good health. Etherization. Semi-circular transversal laparotomy. Evisceration, dissection and extirpation of both normal kidneys, ligation of the lower suprarenal vein, of two collaterals of the vena cava and of the first lumbar pair. Temporary hæmostasis. Section of the aorta about 2 centimeters below the renal arteries. Resection of the venous segment on which are implanted the renal veins. Washing and greasing of the vascular ends. Then gauze compresses with Locke's solution are put on the operative field and protected with a wool blanket.

Extirpation of the Kidneys.—Middle-aged, pregnant cat. Etherization. Opening of the abdomen by the ordinary method. Veins extremely dilated. When the anatomical specimen, both kidneys, and left suprarenal gland are almost completely isolated, the ureters are dissected as far as the bladder. Resection of a flap of the vesical wall around their mouths. Then section of the uterus, mesocolon and the small intestine near the cæcum. The ureters and the flap of the bladder are removed from the lower part of the abdomen and placed temporarily below the left kidney. Perfusion of both kidneys with Locke's solution at the temperature of the laboratory (37° C.). The anatomical specimen is removed and placed immediately in the abdomen of the host.

Transplantation of the Kidneys.—Each kidney is placed under the peritoneum in its normal location. Anastomosis of the vessels and reëstablishment of the circulation. No leakage. It is noticed that the lower end of the venous segment has been twisted and that it produces a very marked stenosis just above the lower anastomosis. It does not interfere with the circulation of the kidney, the color of which almost immediately becomes normal. The circulation has been interrupted for 42 minutes only. Immediate secretion of clear fluid.

The ureters are placed in the abdominal cavity on the right side of the rectum. Longitudinal opening of the bladder on its posterior face and on the middle line, and graft of the transplanted flap of bladder by muco-mucous and musculo-muscular sutures. Closing of the lumbar peritoneum. Through the lower part of this line of suture a small opening is reserved to allow the ureters to pass from the retro-peritoneal space into the peritoneal cavity. Both ureters are twisted around each other, but, as there is no tension, it probably does not interfere with the flow of urine.

The intestines are put back into the abdominal cavity. Suture of the abdominal wall. Dressing. Shirt.

June 20. Animal in good condition. Urine a little bloody. Does not eat.

June 21. Animal in excellent condition, urinates abundantly, eats a great deal of raw meat.

June 22. Animal apparently in very good health, climbs on roof.

June 23. Dressing is removed, wound completely healed. Animal eats meat and drinks a great deal of milk, urinates abundantly.

June 24. Animal looks well, but refuses to eat.

June 25. Animal in good condition, does not eat. Does not vomit. No feces. Clear, yellow urine, urea 2.2 per cent. A little albumin, less than 0.25 g. per 1000 c.c.

June 26. Animal in good condition, walks, climbs on a wall more than six feet high, but refuses to eat and to drink milk. Then a direct examination of the abdominal cavity is decided upon.

10 a. m. Etherization. Longitudinal laparotomy on the middle line. No intestinal adhesions to the abdominal wall. A few adhesions between the duodenum, the anterior face of the right kidney and the inferior face of the liver. They are loose and easily detached. The kidneys are covered by normal peritoneum and apparently in excellent condition. Size, color and consistency normal. The surface of both kidneys is rosy, there is no apparent vasodilatation. No sclerosis of the connective tissue between the kidneys. The renal veins and the transplanted segment of vena cava appear to be absolutely normal. Normal pulsations of the aorta and the renal arteries.

Then closing of the abdominal wound by four planes of sutures.

One hour after the operation, the animal walks and seems in good condition.

June 27, 8 a. m. Animal found dead in its cage. Body is still warm.

Autopsy.—9.15 a. m.

Macroscopical Examination.—Peritoneum normal, no fluid, no adhesions. Bladder filled with urine.

Both kidneys are in their normal location, covered with sound peritoneum, and normal in color, size, consistency and connections with surrounding structures. From a point corresponding to the lower end of the incision of the lumbar peritoneum, both ureters adherent to one another, penetrate into the peritoneal cavity and go down toward the bladder along the right side of the rectum. Excellent union of the transplanted flap of bladder to the bladder of the host (Plate XIII).

Incision of the peritoneum and dissection of the kidneys. Their appearance is normal. They are surrounded by their covering of connective tissue, which does not present any sclerosis. Dissection and incision of the aorta and vena cava. Anastomoses healed without any deposit of fibrin. Vena cava is obliterated 1 centimeter above the lower anastomosis at the point of the twisting of the transplanted venous segment. This point is much below the mouth of the renal veins and the obliteration did not interfere with the venous circulation of the kidneys. Both kidneys are opened. Capsules normal. No hydronephrosis. In section they have the appearance of normal kidneys. A piece of the right kidney is fixed in Zenker's fluid.

Lungs, heart, liver, spleen apparently normal. No examination of the brain and medulla.

Microscopical Examination.—Glomeruli normal. No exudate in the glomerular space. Epithelial cells of the tubules generally well-preserved, brush border very apparent, regularly disposed and adherent to the basement membrane. In some places vacuolization of the protoplasm around the nucleus. Exudate in the lumen of the tubules. Epithelial cells of the excretory tubules normal. A few typical hyalin casts. No infiltration whatever of the intersitial tissue.

Experiment 5.—June 28, 1907. 7.30 a. m.

Preparation of the Kidneys of the First Animal.—Middle-sized female cat in good health. Etherization. Exposition and isolation of both kidneys, which are abnormally pale, the left suprarenal, the ureters and the corresponding part of the bladder by the ordinary method. The aorta and vena cava are dissected but not cut. The organs are covered with compresses of Locke's solution.

Preparation of the Host.—Strong, slender, young, black cat. Etherization. Exposition and extirpation of both kidneys by the ordinary method. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta 2.5 centimeters below the mouth of the renal arteries, resection of the segment of vena cava on which the renal veins are implanted, washing and greasing of the vascular ends.

Extirpation of the Kidneys from the First Animal.—Washing of the kidneys by the ordinary method. Section of the vessels. The anatomical specimen is removed and placed in the abdominal cavity of the second animal. The first animal is killed by opening the diaphragm and clamping the heart.

Transplantation of the Kidneys.—The kidneys are put in their normal location and the vascular segments interposed between the ends of the aorta and vena cava. Anastomoses. Because the incision of the abdominal wall is too short, and consequently the operative field narrow and deep, the anastomoses are difficult and a dissecting forceps is frequently used in the handling of the vascular ends. Reëstablishment of the circulation 45 minutes after clamping the aorta and interrupting the circulation through the kidneys. After a few minutes the kidneys assume their normal appearance; their surface is rosy without white or blue spots. Opening of the bladder and graft of the flap by two planes of sutures.

Suture of the lumbar peritoneum. Through the lower part of the line of suturing an opening is left for the ureters. The intestines are replaced in the abdominal cavity. End of the operation as usual.

June 29. Animal in excellent condition, urinates abundantly.

June 30. Same condition.

July 1. Slight paresis of the posterior limbs.

July 2. Complete paralysis of the posterior limbs and the tail.

July 3. Animal died.

Autopsy.—Peritoneum, kidneys and all abdominal organs apparently normal. Excellent healing of the flap of the bladder. The bladder is distended with urine. Perfect healing of the anastomoses of the vena cava. The upper aortic anastomosis and the renal arteries are in excellent condition. At about 1 centimeter below the mouths of the renal arteries, the aorta is completely obliterated by a clot. This clot is not adherent to the wall of the transplanted segment or to the line of suture. It is fixed to a wound of the intima of the lower end of the aorta at 1 millimeter below the anastomosis. The wound has been caused almost certainly by the handling of the vessel with the dissecting forceps during the performance of the anastomosis.

As the autopsy was performed a long time after death and the temperature was high, the specimens of kidneys are so poorly preserved that no interpretation of the sections is possible.

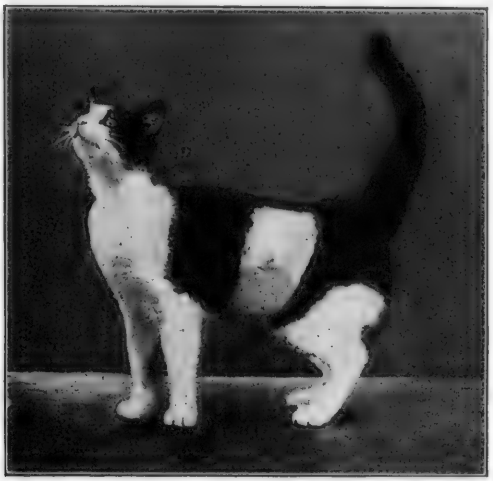
Experiment 6.[13]—July 12, 1907, 8.15 a. m.

Extirpation of the Kidneys.—Black, pregnant cat, mangy and in bad health. Etherization, extirpation and dissection by the ordinary method, both kidneys, left suprarenal gland, ureters and a large flap of bladder. Clamping of the aorta below the diaphragm at 8.40 a. m. Washing of both kidneys with Locke's solution at the temperature of the laboratory. The perfusion is stopped when there is still a good deal of blood in the kidneys and the veins are still filled with bloody fluid. Then the vessels are cut, the specimen removed and put into Locke's solution.

Preparation of the Host and Transplantation of the Kidneys.—White and black, young female cat in excellent health. Bloody discharge from the vagina. Parturition two days before. Etherization difficult. Semi-circular transverse laparotomy. Evisceration of the intestines, spleen and bladder. Longitudinal incision of the lumbar peritoneum, extirpation of both kidneys, dissection of the

[13] This experiment was reported by Mr. R. D. McClure before the Seventh International Zoological Congress.

aorta and vena cava, and temporary hæmostasis. Resection of the segment of vena cava where the renal veins are implanted. Section of the aorta 2 centimeters below the renal arteries. The anatomical specimen is put into the abdominal cavity, the kidneys in their normal location, and the vascular segment between the ends of the aorta and vena cava. Reëstablishment of the circulation at 9.45 a. m. No leakage. In a few minutes the circulation of both kidneys is apparently normal. The ureters are put along the right side of the rectum through the basis of the right broad ligament. Incision of the bladder and implantation of the flap of bladder by two planes of sutures. Suture of the incision of the lumbar peritoneum. The intestines and the spleen are put back into the peritoneal cavity. Intestines cold, a little shock. Suture of the abdominal wall by the ordinary method. Gauze and cotton dressing. Shirt. Animal in a metabolism cage.



Cat 6 looking at a piece of meat. Photograph taken on the twenty-first day after the operation.

July 12, 2 p. m. Animal a little shocked, lies down in its cage.

July 13. Animal lies down and refuses to eat. From time to time she gets up, turns around in the cage, and cries as though suffering abdominal pain. Does not vomit. After having urinated she is quiet and looks comfortable again. Urine 120 c.c.

July 15. Animal much better. Walks about the cage, no abdominal pain. Eats a great deal of raw meat, drinks milk, urinates abundantly. Bloody discharge from the vagina. No analysis of urine.

July 16. Animal in normal condition, walks, jumps, climbs, eats a great deal of meat, drinks milk, and urinates abundantly.

July 17, 18. Same condition.

July 19. Animal in perfect health, is growing fat. The dressing is removed. Wound completely healed. Both kidneys normal in size and situation.

July 20, 21 and 22. Same condition.

July 23. Animal fat, and in good health. Eats a great deal of meat. Both kidneys are a little increased in size and less movable.

July 24. Same condition.

July 25. Animal is apparently normal, runs about the roof, climbs and jumps on the table, eats a great deal.

ANALYSIS OF URINE: EXPERIMENT 6.

	Quantity.	Color.	Reaction.	Density.	Urea per 100 c.c.	Albumin per 1000 c.c.	
July 13	120 c.c.	Yellowish pale	Acid	1.007	1.2	less than 0.5	
14							Mixed with feces and milk.
15							
16	140 "	Yellowish	Acid	1.015	1.7	0.5	
17	210 "	Yellowish		1.019	from 2.7–4.2	less than 0.25	
18	95 "	Yellow	Acid	1.018		less than 0.5	
19	82 "	Yellow		1.021	4.9	less than 0.25	
20	120 "	Yellow	Acid	1.026	4.1	none	
21	145 "	Yellow	Acid	1.020	4.4	none	
22	95 "	Yellow		1.022		none	
23	164 "	Yellow		1.029	5.1	none	
24							Mixed with feces.
25	170 "	Yellow		1.035		traces	
26	60 "	Yellow	Acid	1.030		0.75	
27	175 "						Mixed with feces or milk.
28	185 "						
29	255 "	Yellowish		1.019			
30	165 "	Yellowish pale		1.013	1.8	1.25	
31	215 "					1.2	
Aug. 1	160 "						
2	165 "						
3							
4							
5							
6							
7	from						
8	120–160 c.c.						
9							
10						A great deal of albumin.	
11							
12							

I am much indebted for this observation to Dr. Levene and Dr. Auer who, during August, had the kindness to analyze the urine of this animal and to examine her clinically.

July 26, 27, 28, and 29. Same condition.

July 30. Animal is apparently in excellent health. Nevertheless, both kidneys are enlarged and increased progressively in size and are completely fixed and adherent to the lumbar region. They are no longer movable in the abdominal cavity as normal kidneys are.

July 31 and August 1. Same condition.

August 2. A photograph is taken while the animal is eating. (See figure.)

August 3. Animal is apparently in good health, but the kidneys are enlarged and the urine contains more albumin.

From August 3 to August 10 the animal was in excellent condition, eating and acting as a normal cat. On August 11 she began to vomit and in a few hours became very ill. A great deal of albumin in the urine. Died on August 12.

Autopsy.—The body was opened by Mr. McClure. The peritoneum, the intestines, the lungs and the heart were found normal. The kidneys and the vessels were not examined, but the body was put in formalin, and the autopsy completed on October 4.

Macroscopical Examination.—Kidneys in their normal location and greatly enlarged. Normal cicatrisation of the flap of the bladder. Both kidneys are strongly adherent to the posterior abdominal wall and to each other. They are united by a growth developed under the peritoneum and passing as a bridge over the aorta and vena cava. This growth is developed outside of the capsule of the left kidney. It is adherent to and interposes itself between the renal vessels, the aorta and vena cava. The vena cava is compressed against the internal face of the right kidney. The right renal vein is also compressed between the tumor and the kidney, while the left renal vein is too much extended. This growth is composed of hard, white, apparently fibrous tissue, well-defined on its anterior side and more diffuse on its posterior side. It is intimately adherent to the transplanted suprarenal gland. Anastomoses excellent. Opening of the kidneys. No hydronephrosis. Congestion. Dilatation of the stellate veins.

Microscopical Examination.—Cadaveric changes of the epithelial cells are so marked that an interpretation of the epithelial lesions is not possible. Glomeruli well preserved, dilatation of the capillary loops which fill almost completely the capsules. Infiltration by plasma cells of the interstitial tissue. This lesion is more marked in the cortex than in the medulla. Focal disposition. Very marked dilatation of the blood-vessels. Appearance of acute interstitial nephritis. Section of the growth shows organized blood clot.

Experiment 7.—July 17, 1907.

Extirpation of the Kidneys.—Pregnant female cat. Etherization. Preparation by the ordinary method of both kidneys, left suprarenal gland, vessels, ureters and flap of bladder. Incomplete perfusion of the kidney as in the previous experiment. Extirpation of the anatomical specimen, which is put into Locke's solution at the temperature of the laboratory (22.2° C.).

Transplantation of the Kidneys.—Young, black, pregnant cat in excellent health. Abdomen considerably enlarged; near the end of pregnancy. Semicircular transversal laparotomy. Evisceration of the intestines, the spleen, the bladder filled with urine, and the uterus, which is distended by several fœtuses. Ligation of the left ovarian vein. Longitudinal incision of the lumbar peritoneum

in the middle line and extirpation of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis with *serre fines.* The anatomical specimen is placed in the abdominal cavity by the ordinary method. Reëstablishment of the circulation 60 minutes after the interruption. No leakage. Excellent and quick reëstablishment of the circulation through the kidneys which assume almost immediately a normal color. Incision of the bladder and graft of the flap of bladder by the ordinary method. After completion of the suture, it is observed that the ureters are twisted around each other. However, the transplanted ureters being very long there is no tension and it will not interfere, probably, with the flow of urine. The intestines, the spleen and the uterus are put back into the abdominal cavity, which is closed by the ordinary method. Gauze and cotton dressing. Shirt.

July 18. Animal lies down, urinates abundantly and looks in good condition.

July 19. Parturition normal. No eclampsia. Urinates and is in good condition.

July 20. Animal apparently normal, walks, drinks milk and eats meat. Bloody discharge from the vagina.

July 21, 22, 23, and 24. Almost the same condition.

July 25, 26. Animal less well, urinates and eats a little meat. High temperature.

July 27, 28. Animal refuses to eat, and looks very ill. However, she urinates abundantly. Bloody discharge from the vagina.

July 30. Animal died.

Autopsy.

Macroscopical Examination.—Large abscess of the pelvis, located on the left side, between the pelvis and the rectum, extending as far as the subperitoneal space, and opening to the skin near the anus, on the left side. Big and soft uterus. The left uterine horn is increased in size. On section reddish fluid and a part of a placenta is found.

No peritonitis. Intestines normal. Both kidneys surrounded by loose connective tissue, normal in location, color and consistency. Anastomoses and vessels normal. Slight congestion of the pyramids. No dilatation of the ureters. Excellent healing of the bladder.

Microscopical Examination.—Glomeruli well preserved. No coagulated fluid between the capsules and the capillary loops. It is difficult to appreciate exactly the lesions of the epithelium of the tubules, for the fixation of the specimen is not good. It seems well enough preserved. Slight leucocytic infiltration between certain tubules. Slight dilatation of the blood-vessels.

Experiment 8.—July 19, 1907.

Extirpation of the Kidneys.—Middle-aged pregnant cat. Dissection of the anatomical specimen by the same method as in the preceding experiments. A small quantity of Locke's solution at the temperature of the laboratory (31° C.) is injected into the kidneys. The washing is stopped when the renal vein is still filled with bloody fluid. Anatomical specimen removed and put in Locke's solution at the temperature of the laboratory. Cat killed by hæmorrhage.

Transplantation of the Kidneys.—Long, middle-aged male cat. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen. Extirpa-

tion of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta below the renal arteries. Section of the vena cava between the openings of the renal veins. The kidneys are removed from their jar, put into the abdominal cavity and the vessels anastomosed by the ordinary method. Reëstablishment of the circulation 1 hour and 5 minutes after the interruption. No leakage. Excellent circulation through the kidneys, which assume, after a few minutes, their normal appearance. Opening of the bladder and graft of the flap of bladder. Suture of the lumbar peritoneum. It is found that the right ureter goes behind the vena cava and produces a slight degree of compression of this vessel. The intestines are put back into the abdominal cavity. Closing of the abdominal wall by three planes of sutures. Gauze and cotton dressing. Shirt.

July 20. Animal in excellent condition. Urinates abundantly.

July 21, 22. Animal drinks milk.

July 23. Animal apparently in normal condition, walks about the roof. The dressing is removed. Apparent healing of the wound.

July 24. Same condition.

July 25. Animal eats raw meat.

July 26, 27 and 28. Animal in good condition, eats meat.

July 29. Animal is less well. An abscess has developed on the left side below the end of the abdominal suture.

July 30. Animal looks ill. However, he urinates as usual.

July 31. Animal refuses to eat, and looks very ill. The abscess is examined. It is found that it is an extensive and deep abscess of the wall itself, and not merely of the subcutaneous tissue. All the left part of the abdominal wall is involved. Large openings and drainage. In the evening the animal is worse.

August 1. Animal died in the morning.

Autopsy.

Macroscopical Examination.—Large abscess infiltrating the anterior and left parts of the abdominal wall. No peritonitis. Heart, lungs, liver, and spleen normal. Both kidneys are almost normal in size, color, and consistency, and surrounded by a layer of adipose tissue, which has become very abundant below the lower end of the kidneys. Their mobility is normal. Both ureters adherent to one another enter the peritoneal cavity at the level of the lower end of the incision of the peritoneum near the lower end of the left kidney. Perfect union of the transplanted flap to the bladder.

Opening of the bladder. On the posterior wall the flap is found limited by a linear and almost circular scar. The transplanted mucous membrane is normal. The mouth of each ureter presents its ordinary appearance.

Opening of the lumbar peritoneum and dissection of the vena cava. No perivascular sclerotic tissue. The right ureter is twisted around the vena cava. Renal veins almost horizontal, dense connective tissue around the left suprarenal gland. Opening of the vena cava, anastomoses perfectly smooth. A little clot around and in which a short piece of silk thread is found free in the lumen of the vein at 1 centimeter above the upper anastomosis. In a point of the upper anastomosis is a very small red spot where this clot was probably adherent. Dissection of the aorta. The lower anastomosis is almost invisible: at the level of the upper anastomosis, the aorta is adherent to the vena cava by

dense connective tissue. This tissue is removed and, corresponding to the point where it is mostly adherent to the wall of the aorta, a very small opening of the line of sutures between two stitches is observed. The aorta is opened: anastomoses smooth and glistening.

Longitudinal section of the kidneys: cortex and pyramids almost normal although a little congested. A small piece is fixed in Zenker's fluid.

Microscopical Examination.—Glomeruli well preserved. Epithelium of the tubules without very marked lesions. Very few casts. Extensive foci of infiltration of the interstitial tissue by plasma cells; subacute interstitial nephritis. Slight dilatation of the blood-vessels.

Experiment 9.—October 14, 1907.

Dissection and Preparation of the Kidneys.—Middle-aged female cat. Etherization. Preparation of the anatomical specimen as usual. The lower suprarenal vein is ligated and the gland is not included in the specimen. Section of the uterus and the intestine. The specimen, of which the circulation is not interrupted, is protected by the omentum and towels.

Preparation of the Host.—Gray and white female cat, living in the laboratory several months; young and in good health. October 1. Urine examined—yellow, clear urine. Density 1.039. Urea 5.9 gr. per 100 c.c. No albumin. Etherization. Opening of the abdomen and evisceration as usual. The intestines are protected by Japanese silk towels sterilized in vaseline. Dissection and extirpation of both kidneys. Ligature of the ovarian veins, of the lower suprarenal vein and of two aortic collaterals.

Extirpation of the Kidneys.—Clamping of the aorta below the diaphragm at 11.25 a. m. Incomplete washing of the kidneys with Locke's solution. Section of the vessels and removal of the specimen.

Transplantation of the Kidneys.—The specimens are placed immediately in the abdominal cavity of the host. After temporary hæmostasis with *serre fines*, the aorta is cut 3 centimeters below the renal arteries. Resection of the region of implantation of the renal veins. Washing and greasing of the vascular ends. Anastomosis of the vessels. Reëstablishment of the circulation at 12.15 p. m. No leakage. Excellent circulation through the kidneys and the small vessels of the lower ends of the ureters. Nevertheless, the left kidney remains pale, while the right kidney is rosy and secretes urine. Opening of the bladder and graft of the flap by two planes of stitches. After the completion of this suture, a slight hæmorrhage from the lower arterial anastomosis is noticed and controlled by one stitch.

The appearance of the kidneys is now very much modified. The left kidney is vasodilated, and its vein carries red blood, while the right kidney has become pale and vasoconstricted, and its vein is filled with dark blood.

End of the operation as usual.

October 14, 4 p. m. No shock. Animal walks, drinks water, has urinated 9 c.c. of bloody urine, the blood depositing itself quickly at the bottom of the glass, urine clear on the top. Urea 4.9.—11 p. m. Urinates again, much less blood.

October 15. Animal a little sick, drinks water, walks about the cage.—4 p. m. Since yesterday 25 c.c. urine only, dark yellow, with a little blood. Albumin. Density 1.051.

October 16. Animal in better condition, drinks milk and eats a little meat. Urine 16 c.c.

October 17, 18. Good condition, drinks milk, is given very little meat.

October 18. Same condition.

October 19. Cat in excellent condition. He is given plenty of milk and very little meat.

October 20. Same condition.

October 21. Cat is completely recovered. He is a normal cat, in the same condition as before the operation.

October 22. Diet from now raw liver and milk.

October 23. Dressing is removed; wound completely healed.

October 24. Cat normal. By palpation the kidneys are found small and movable. They are apparently normal.

October 25. A sample of urine is examined. Clear, yellow urine, acid, density 1.030. Urea 5.1. No albumin.

October 26. Cat normal, kidneys normal in size and movable.

October 27. Same condition.

October 28. Cat being normal is allowed to go out from the cage and to run freely through the room.

October 29. Cat is put into another room and spends all day climbing on and jumping off the furniture.

October 30. Normal general condition, kidneys a little enlarged and less movable.

October 31. Cat a little depressed. The kidneys are very much enlarged and fixed to the lumbar wall. Samples of urine are then examined.—1 p. m. Pale yellow, clear urine. Urea 3.6. Marked quantity of albumin.—3 p. m. Heavy precipitate of albumin by nitric acid.—6 p. m. Albumin 6 gram per 1000 c.c.

November 1. Cat is a little depressed, but still in very good general condition. The kidneys are much enlarged. Very large amount of albumin.—10.30 a. m. Etherization. Semi-circular transversal laparotomy, just above the scar. A few adhesions of the omentum to the wall. Peritoneum and intestines normal. Both kidneys appear very much enlarged and covered with sound peritoneum. Their consistency is a little softer than normal. Incision and dissection of the lumbar peritoneum on the middle line. There is a little sclerosis of the subperitoneal connective tissue around the anterior side of the vena cava. It may possibly produce a slight degree of compression of the vessel. Nevertheless, the arterial and venous circulation appear to be normal. The connective tissue of the hilus is œdematous, clear fluid flows after incision. The wall of the ureter is œdematous, without congestion. The small vessels are distinctly seen with red blood. The color of both kidneys is rosy and normal. There is no congestion. Incision of the capsule of the right kidney: clear fluid and red blood flow. The tissue of the kidney is incised: it is œdematous and not congested. Abundant hæmorrhage. Suture of the capsule with Lyon's silk and needles, No. 16. Both renal veins of the right kidney are dissected. The circulation is normal. The perivascular connective tissue is not sclerotic but œdematous. During the dissection of the upper vein several blue, round spots are seen on the surface of the organ, and disappear after a few minutes. No suture of the incision of the lumbar peritoneum. Abdominal wound closed as usual.

6.30 p. m. Cat in good condition. Urine 18 c.c. Much less albumin, 2.75.

November 2, 8 a. m. Cat in good condition, but depressed. Walks about cage. Takes milk only, possibly a little meat.—10.30 a. m. Urine 19 c.c. Albumin 0.60.

9 p. m. Urine 53 c.c. Density 1.020. Albumin 1.50. Urea 4.2.

November 3. Same condition. Diet consists only of milk. Cat depressed.

November 4. Better condition. 8 a. m. Urine 61 c.c., dark yellow, density 1.033. No albumin.

November 5. Same condition.

November 6. Albumin again 1.50.

November 7. Cat depressed, walks about its cage.

November 8. Better condition. Eats a little fish.

November 9. Cat in better condition, but very emaciated. Therefore, he is given rare liver and codfish and eats hungrily. Dressing removed. Wound healed. Both kidneys much diminished in size, but still abnormally large.

November 10. General condition improved.

November 11. Both kidneys diminish steadily in size. Cat eats codfish.

November 12. Cat eats meat and fish. From 9 a. m. to 6.30 p. m., urine 48 c.c., clear yellow. Density 1.021. Urea 3.2. Albumin 1 gr.

November 13. Cat in good condition. Albumin less than 1 gr.

November 14. Cat well in the morning. In the evening, looks ill. Discharge from the nose. Refuses to eat.

November 15. Abundant nasal discharge. Cat refuses to eat. Urine, yellow clear. Albumin less that 1 gr.

November 16. Animal weak and emaciated. Very abundant purulent nasal discharge. The quantity of urine during the last twenty-four hours was 78 c.c., yellow. Density 1.031. Albumin 1.80. Urea 4.8. Many red blood corpuscles and granular casts. The size of the kidneys almost normal.

November 17. Animal very emaciated, weak, but still able to jump from his cage and walks about the room. Abundant nasal discharge. Refuses to eat.

November 18. Animal very weak.

November 19. 1 p. m. died. Post-mortem 2.15 p. m. Opening of the abdominal cavity. A loop of jejunum is adherent to the posterior abdominal wall. Sharp flexure without obstruction. Both kidneys normal in location, color, size and consistency. Ureters normal. Perfect healing of the transplanted flap of bladder. In the bladder, yellow clear urine 5 c.c., albumin. Longitudinal incision of the kidneys, which are apparently normal. However the cortex is pale. Medulla normal. Capsule normal. No dilatation of the stellate veins.

The main pathological change is a general and intense calcification of the arterial system. The arteries are as hard and friable as thin-walled glass tubes. These lesions have developed since the transplantation, for the cat was a young animal in excellent health and its abdominal aorta was perfectly normal.

V. RESULTS.

The results of the experiments will be examined from the clinical and anatomical standpoints.

A. *Clinical Results.*

In every case the reëstablishment of the renal functions was observed. These functions were determined by the characters of the urines and the general condition of the animals.

The secretion of urine may begin as soon as the arterial circulation is reëstablished. In several cases, clear urine flowed from the ureters while the flap of bladder was being grafted onto the host. More often, no urine was seen flowing from the ureters immediately after completion of the operation. But the secretion always began during the first twenty-four hours. It is difficult to ascertain exactly the amount of urine secreted during the first few hours, because of the vomitus and water which are often mixed with urine. However, Cat 6 did not vomit, and after the first twenty-four hours the jar contained 120 cubic centimeters of urine. On the other hand Cat 1 urinated very little on the first day. Cat 9 urinated only 25 cubic centimeters during the first twenty-four hours; the second day the amount of urine passed was only 16 cubic centimeters, this urine was highly concentrated and contained much urea. These differences in the immediate amount of urinary secretion are due probably to unknown conditions of the vasomotor nerves. It is generally supposed that denervation of the kidney produces the secretion of an abundant and diluted urine. In the simple transplantation of the kidney, when, for instance, an isolated organ is transplanted into the neck, these phenomena were observed. But in the case of transplantation in mass, immediate vasodilatation is not so marked. Sometimes there is vasoconstriction, but oftener the kidneys retain their normal appearance. Exceptionally, vasodilatation alternates with vasoconstriction. In Experiment 9 about ten minutes after the reëstablishment of the circulation, the right kidney was rosy, its venous blood red, and some urine flowed from its ureter, while the left kidney was pale, and apparently did not secrete. About thirty minutes afterwards, when the suture of the bladder was completed, both kidneys were examined again. The conditions were now reversed: the right kidney had become pale, and its vein filled with dark blood, while the left kidney was rosy and its vein contained red blood. It seems

that following transplantation the renal ganglia begin to act and that variable conditions of the nervous system may be responsible for the differences in the immediate results observed.

In all the experiments, the urinary secretion went on as long as the animal lived. Every cat urinated abundantly every day. But the animals presented sooner or later some complication, which modified in some measure the renal functions. As is to be expected after an operation as complex as the transplantation in mass, various accidents occurred; hydronephrosis, intestinal compression by peritoneal adhesions, volvulus, phlegmon, puerperal infection, compression of the renal veins by organized hæmatoma of the connective tissue, which were the direct or indirect causes of death in these animals. It is well known that several of the complications, especially the compression of the renal veins, produce grave renal lesions of their own. Therefore, the results of our experiments must not be considered as expressing generally the normal condition of transplanted kidneys, but merely of transplanted kidneys when subjected to various complications, that is, of more or less abnormal transplanted kidneys. Actually, it is impossible to know exactly how a normal transplanted kidney would functionate, for we cannot as yet discriminate between the disorders produced by such a common complication as hydronephrosis or compression of the veins and the less defined ones which may be due to lesions produced by the transplantation itself. However, in Experiment 6 and 9, for instance, the functions of the kidneys seem to have been for a certain time almost completely normal.

The color of the urine was yellow, generally or often less dark than the normal urine of the cat. Its reaction was acid. Its quantity for twenty-four hours oscillated between 120 and 160 cubic centimeters. But it might be, exceptionally, 25 and even 15 cubic centimeters, or, in another case, 215 or 255 cubic centimeters for twenty-four hours. In this case there was congestion of the kidneys produced by venous compression. The density was very far from constant; generally it oscillated between 1.018 and 1.030, going sometimes as high as 1.035 and 1.051. In Experiment 6 there was little parallelism between the amount of urine and the

density. Once the kidneys secreted 170 cubic centimeters of urine with a density of 1.035.

In all cases the amount of urea bore a relation to the diet of the animal. In Cat 6, abundantly fed with raw meat, the amount of urea varied from 2.7 to 5.1 grams. Cat 9 passed through his own kidneys, fourteen days before the operation, 5.9 grams of urea for 100 cubic centimeters. Eleven days after the operation, he eliminated through his new kidneys 5.1 grams of urea for 100 cubic centimeters. The difference is explained by the diet which was less abundant after the operation than before.

Among the abnormal constituents of the urine, the presence of albumin only has been looked for. In Experiment 1 albumin was present during the fourteen days of the post-operative life of the animal. These kidneys were abnormal owing to the perfusion with too hot Locke's solution and to a developed hydronephrosis. In the other cases there was little albumin during the first days, ranging from 0.50 to 0.25 gram for 1000 cubic centimeters. This was probably due in part to the blood coming from the suture of the ureter or the bladder. The amount of albumin decreased progressively and disappeared about one week after the operation. In Experiment 6 albumin was again found on the thirteenth day after the operation, and its amount increased progressively to 1.50 grams and beyond. In Experiment 9 there was albumin in the urine one day after the operation. On the eleventh day no albumin at all was present. On the fifteenth and sixteenth days the animal was allowed to run and climb freely. On the seventeenth day albumin was found again in marked quantity and, at the same time, enlargement of the kidneys was distinctly detected by palpation.

The general condition of the animal can be used, in some measure, to indicate the perfection of the urinary elimination. As long as no complications were present, the animals lived as normal cats do, without presenting any symptoms which could be considered as produced by renal insufficiency. When general complications occurred the cats reacted against them in normal ways.

Cat 1 suffered from eventration, due to a premature resorption of the catgut in the abdominal suture. In two other animals sutured with the same catgut, the resorption and eventration

occurred on the fifth or on the seventh day after the operation. We may admit that in Experiment 1 the resorption took place at about the same time. However, in spite of the extrusion of intestine outside of the abdominal cavity in the gauze dressing, the animal lived several days, drank water and milk and ate a little meat, and urinated abundantly. When the reduction of the inflamed intestines into the abdominal cavity was performed, fourteen days after the operation, the animal was still able to overcome the operative shock, and to walk about the room a few hours later.

In Experiment 6 the animal was in apparently normal condition four days after the operation. She walked about the room, played and ate a great deal of raw meat. Her condition remained excellent for several weeks. Twenty days after the operation she was in good health, had glossy hair, was very fat and ate with appetite all kinds of food. She ran about the room, played, jumped and climbed on the desks and tables as a normal cat does (see figure). There was, however, albumin in the urine, and slow and progressive enlargement of the kidneys took place, which showed that she was not in an entirely normal condition. Nevertheless, until the twenty-ninth day after the operation, she seemed to be in excellent health. Then gastro-intestinal symptoms appeared and death occurred on the thirty-first day after the operation. In Experiment 7 the animal operated on was a pregnant cat whose uterus contained several large fœtuses. After the operation, she was immediately in good condition. Two days afterwards parturition occurred without any eclamptic fits or any abnormal symptoms. As the animal seemed to recover very easily and began eating meat one day afterwards, she was not observed very carefully. The dressing was removed and she was let alone. When she was examined again, eight days after the operation, she was found less well and feverish. Her condition grew worse and she died thirteen days after the operation. The autopsy showed puerperal infection with retention of a placenta and an enormous abscess of the pelvis.

Experiment 9 was a female cat which lived in the laboratory for several months. She was in excellent condition when she was operated on, and recovered very quickly from the operation. Her life went on just the same as before. The kidneys were movable

and small. She looked in excellent health and lived as a normal cat. On the eighteenth day after the transplantation a direct examination of the kidneys was made to ascertain the cause of the appearance of albumin. The general condition was little affected by the operation and the albumin disappeared on the twenty-first day, but reappeared again a little later. On the thirty-fifth day, the animal was very weak and emaciated. She died on the thirty-sixth day.

We can conclude from these results that the functions of the kidneys reëstablish themselves after the transplantation. Since an animal, such, for instance, as Cat 6, can live in an apparently prosperous condition of health fifteen or twenty-five days, and more, after a double nephrectomy, and eliminate each twenty-four hours 120 and 160 cubic centimeters of urine through the new kidneys, it is certain that the functions of the transplanted organs are efficient. Even these functions during a part of the life of animals No. 6 and 9 can be considered as having practically been normal. When complications contingent or inherent to the actual method of transplantation occurred, the functions of the kidneys were modified and became abnormal according to the pathological changes suffered by the organs.

B. *Anatomical Results.*

The Blood Vessels.—The condition of the blood vessels was examined three times by laparotomy on the living animal and in the other cases at the autopsy.

The direction of the vena cava was almost always found normal. Once, however, the interposed segment was too long and bent. In Experiment 9 the position of the veins was modified considerably by an organized hæmatoma which had pushed the vena cava against the right kidney. The right renal vein was compressed and the left one too much extended. This diminished the activity of the venous circulation and produced marked congestion of the kidneys. It is very important that the veins be given their normal situation and direction. On account of the low pressure and the thinness of the wall, they are not able to take care of themselves as arteries do. Every departure from the normal produces a diminution of

the caliber and consequently slight or marked congestion of the transplanted organ.

The relations of the veins with the surrounding structures, arteries and ureters were generally normal. It happened once that by mistake the right ureter was twisted around the lower part of the venous segment. It did not appear to cause any marked disturbance. The vessels were free in loose connective tissue, excepting in Experiment 6 where the vena cava and renal veins were compressed by an organized hæmatoma. When progressive compression of the veins had occurred it was expressed clinically by progressive enlargement of the kidney, and by reappearance of albumin in the urine on the thirteenth day after the operation, while the animal appeared otherwise in perfect health. In another case there was a little sclerosis of the perivascular connective tissue, which produced some retraction of the right kidney toward the middle line. The induration or sclerosis of the connective tissue may be a serious secondary complication. It occurs in the transplantation in mass but more often in simple transplantation, and oftenest in transplantation with implantation of the renal vessels on the aorta and vena cava. The induration has no influence on the arteries, but interferes with the venous circulation. As soon as the vein is no longer able to dilate freely in the loose connective tissue of the hilus, its circulation is slightly hampered and the result is a chronic congestion of the organ. This sclerosis may be brought about also by slight non-suppurative inflammation or perhaps by chemical irritation. It is probable that this condition is often due to a slight infiltration of blood into the connective tissue. Blood has an irritative influence on tissues. In Experiment 8 there were strong adhesions between the aorta and vena cava, at the level of the upper arterial anastomosis. The vessels were connected by a mass of dense connective tissue. The aorta was dissected, and the maximum of adhesion was found to be on the anastomosis itself and on this point there was a small gap between two stitches. The connective tissue was probably produced under the influence of the infiltration of blood through this opening. Many examples of this sclerotising influence of blood have been observed after transplantation of segments of vessels. It is well

known that extravasation of blood in joints, muscles, or in the central nervous system produce hard connective tissue. Thus injection of blood has been utilized by Bier and Schmieden[14] for inducing callous formation in cases of pseudarthrosis. The perivenous sclerosis, which is a dangerous secondary complication in transplantation of organs, can be prevented probably by rigid hæmostasis and asepsis.

The venous anastomoses healed without thrombosis or stenosis. In one case there was an obliteration of the vena cava due to a torsion of the vein. But this was quite independent of the anastomosis. In Experiment 8 the venous anastomosis was normal. Nevertheless, a very small ovoid clot, which developed around a fragment of silk thread, was found free in the lumen of the vein, about one centimeter above the upper anastomosis. On the anastomosis itself by minute examination a little red spot was detected, which might have been the point where this clot was adherent. This is an absolutely exceptional complication.

The aorta and the transplanted aortic segment assumed in every case a normal direction and appearance. The direction of the renal arteries was the reverse of normal. This was due to the fact that the transplanted segment was fixed on the aorta below the implantation of the normal renal arteries. A modification of direction has no harmful influence on the arterial circulation. The thickness of the wall and the high blood pressure allow the arteries to adapt themselves to abnormal situations. Only one complication was observed: in Experiment 5 there was a complete obliteration of the lower part of the venous segment and the lower end of the aorta by a thrombus. This thrombus was dissected and found adherent to a wound of the intima, just below the lower anastomosis. The wound was evidently produced by the dissecting forceps used in this case for handling the vessel.

The anastomoses healed without thrombus or stenosis. The intima of the transplanted segment was smooth and glistening. No deposit of fibrin was observed, and consequently no embolus. In Experiment 1 a fatty embolus was noticed. A few minutes after the reëstablishment of the circulation, the right kidney assumed

[14] Schmieden, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 395.

a rosy and normal color, while the left kidney remained pale. The lower end especially was almost completely yellowish white. An incision through the capsule was made at this point. A small hæmorrhage of red blood mixed with a good deal of vaseline followed, and stopped after a few moments. However, the circulation of the left kidney improved progressively and after thirty minutes was almost normal. It is probable that the vaseline under the progressive increase of temperature of the kidney became more fluid and flowed through the capillaries. The anatomical examination of the kidneys, fifteen days after the operation, showed no evidence of this embolus. The few accidents described could be almost completely prevented by operating on animals of a larger size.

The Nervous System.—The anatomical conditions of the nervous system of transplanted kidneys are not yet known. The attempt was made to preserve as completely as possible the circulatory apparatus of the nervous ganglia with the hope that they would resume their functions partially. It is not impossible to believe that sympathetic ganglia of which the vessels are respected and the circulation reëstablished immediately after the transplantation do not degenerate completely. It has been shown, especially by the experiments of Morat,[15] that sympathetic nerves have some of their trophic centers in the ganglia. A part of the vasoconstrictor nerves of the tongue, contained in the hypoglossus nerve, have a trophic center in the superior sympathetic ganglion. After section and degeneration of the sympathetic, stimulation of the hypoglossus still produces vasoconstriction of the tongue. After extirpation of this ganglion, stimulation of the hypoglossus becomes negative. After intra-cranial section of the facial nerve on a dog, almost all vasomotor fibres degenerate from six to twenty-six days after the operation. However, the stimulation of the chorda tympani produces a slight vasodilatation of the gland. Morat assumes that in this case the geniculatus ganglion must be considered as a trophic center for these fibers.

Even if it be admitted that nervous ganglia can, in some measure, functionate when severed from the central nervous system, it is

[15] Morat, *Comp. rend. de l'Acad. des sciences,* 1897, cxxiv, 1389.

not certain that transplanted ganglia can recuperate their functions. The experiments of Stewart and Guthrie[16] show that after an acute and complete anæmia of the nervous centers for more than twenty minutes, the reëstablishment of their functions is not possible. The functional activity of the ganglia should necessitate also the re-establishment of a practically normal circulation. The experiments of Tuckett[17] have demonstrated that if the vascular supply of the upper sympathetic ganglion is deranged, degeneration sets in forthwith. But, by preserving the connective tissue surrounding the pedicle of the kidneys and not cutting the small collateral branches of the aorta and vena cava, the vascular apparatus of the kidneys can possibly be kept in its integrity. In this condition, the ganglia may resume their functions, if it be physiologically possible.

Ureters and Bladder.—Dilation of the ureters and hydronephrosis took place in the cases where anastomoses of the ureters were performed. In Experiment 3 the upper part of both ureters was very much dilated and the invaginated part stenosed. However, the urine flowed into the bladder satisfactorily, as is shown by the clinical history of the animal. The ureters of a cat are so small that the anastomosis is very difficult, and stenosis or disunion occurs. On small animals it seems proper to give up completely uretero-ureteral anastomoses.

The results of the graft on the bladder of the host of a fragment of bladder extirpated around the points of implantation of the ureters were excellent. The anatomical specimens showed that there was no distension of the upper part of the ureters or the kidneys. Both ureters, adherent to one another, entered the peritoneal cavity through the lower part of the incision of the lumbar peritoneum and went downward along the right side of the rectum. In one case they were twisted around one another; in another, the right ureter was twisted around the vena cava. In spite of these faults of technique the functional and anatomical results were very satisfactory.

In every case, the union of the flap of the bladder of the host took place. After opening the bladder, the transplanted flap ap-

[16] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289.
[17] Tuckett, *Jour. of Physiol.*, 1905, xxxiii, 77.

peared congested and swollen, or entirely normal with the same color and appearance as the surrounding mucosa from which it was separated by a linear scar. On the surface of the transplanted mucosa both openings of the ureters were distinctly seen, normal in size and appearance.

The Kidneys.—The kidneys were examined three times only on living animals. During the operations performed on Cats 1 and 9, fourteen and eighteen days after transplantation, the kidneys were seen covered with sound peritoneum and as regards their color, situation and general appearance, they looked just like ordinary hydronephrotic or œdematous kidneys. In other cases, the anatomical examination was performed after the autopsy. For this part of the work, I am very much indebted to Dr. Simon Flexner, who had the kindness to look over the specimens and histological sections and to give me the invaluable help of his advice.

Macroscopical Examination.—In the experiments where there was no hydronephrosis or venous compression, the size of the kidneys was normal. Hydronephrotic and congested kidneys had their ordinary appearances. Their location was always normal. They remained at the place where they were put during the operation and maintained by the suture of the lumbar peritoneum. As a rule, they were not as movable as the cat's kidneys are normally. However, in Experiments 6 and 7 for instance, their mobility was practically normal. The kidneys of Cat 6 were strongly united to each other and to the lumbar wall by hard connective tissue. In the cases where no hydronephrosis or congestion took place, the consistency of the organ was normal. The external and internal appearance of the organs presented no special characters. They looked like congested, hydronephrotic, or almost normal kidneys. The kidneys of Cat 6 were very much congested. The capsule was slightly adherent to the parenchyma. The stellate veins were very much dilated and the medulla and cortex much increased in size.

In Experiments 1 and 3 the urine flowed from the organs when opened, the calices and pelves were dilated, and the surface of the parenchyma congested. The kidneys were ordinary hydronephrotic

organs. In the other experiments, the kidneys assumed the appearance of normal or slightly congested organs.

Microscopical Examination.—The specimens were generally fixed in Zenker's fluid and stained in hæmatoxylin and eosin. Some of them were taken from the animal several hours after death. In Experiments 6 and 7, the body was simply opened and put in a jar of formalin, while the pieces for histological examination were cut from the kidneys two months after. These faults of technique explain why in several cases the specimens were so badly hardened and why there were such cadaveric changes, especially in the epithelium of the tubuli contorti that an interpretation of the pathological lesions was difficult. It was found that the kidneys presented some lesions, very slight in some cases and more marked in others. In Experiment 4, for instance, the glomeruli and the epithelia of the tubules were very well preserved. There was no interstitial infiltration, and a few casts only were observed. The lesions of these kidneys were very slight.

The lesions noticed in the other experiments belong to two classes; hydronephrosis and nephritis. Hydronephrotic lesions were observed in Experiments 1 and 3. The excretory tubes were very much dilated. There was, too, some dilatation of the tubules of which the epithelium was flattened. In a few places between the tubes slight interstitial infiltration was present. In Experiment 3 the glomeruli were normal. In Experiment 1 some coagulated fluid was seen between Bowman's capsule and the capillary loops. The changes of the epithelium of the tubules were slight. The cells were regularly disposed inside the basement membrane, and the brush border was distinctly seen. The lumen contained some fluid exudate, but very few casts were observed. All these lesions may be explained by the presence of hydronephrosis.

Inflammatory lesions were present in three cases; very slight in Experiment 7, but more marked in Experiments 6 and 8. The epithelial degeneration was not extensive in Experiment 8. It seemed more marked in the other cases, but the cadaveric changes were so pronounced that no accurate interpretation was possible. The characteristic lesion met with in these three cases was the infiltration of the interstitial tissues between the tubules. The most

marked case was Experiment 8, in which the foci of infiltration were extensive. The infiltration was composed of cells having the characters of the plasma cells described by Councilman[18] in acute interstitial nephritis, so-called.

This subacute interstitial nephritis is not a necessary complication of the transplantation, since it was absent from the first cases. It is due probably to secondary causes, physical or chemical conditions of the fluid used in the perfusion, congestion of the organ, diet, general condition of the animal, etc. Many factors may come into play during and after the transplantation for injuring the kidneys. The interstitial and epithelial lesions are due doubtless to some of these. It was regarded as surprising that comparatively few changes were found in the renal structure considering that the organs had been exposed to the rough handling of the transplantation. The cells of the secretory epithelium of the kidney are extremely delicate and sensitive to the modifications of the circulation, etc. It is well known that temporary ligature of the renal vein produces extensive degeneration of the epithelial cells. The simple suspension of the circulation has a harmful influence on the epithelium. The cadaveric disintegration of the cells of the tubules begins very early. One hour after death, the brush border has almost always disappeared.

In the transplantation, the renal tissue is not only deprived of circulation for one hour at least, but is also subjected to a perfusion with a fluid which exerts probably its own harmful influence. The perfusion of the organs seems necessary for preventing the formation of clots and the occurrence of thrombosis of the vessels or infarcts of the kidneys. The solution employed is the ordinary Locke's solution. It has been chosen because it is a physiologically balanced fluid. Pure sodium chloride solutions have injurious effects on the tissues. Ringer has shown that minute amounts of calcium and potassium salts antagonize the effects of the pure sodium salt. Loeb[19] and his pupils have laid special emphasis on the poisonous effects of pure sodium chloride solution. Fundulus eggs put in a pure sodium chloride solution of the same concentration as sea

[18] Councilman, *Jour. of Exper. Med.*, 1898, iii, 393.
[19] Loeb, *Amer. Jour. of Physiol.*, 1899–1900, iii, 327.

water cannot live, but they can live if a definite proportion of calcium chloride be added. Howell[20] and Harvey Cushing[21] also showed the injurious effects on the heart and muscles of the pure sodium chloride solution. In their experiments on tumors, Flexner and Jobling[22] found that the percentage of successful transplantations is much higher when the fragments of tumor have been preserved in Ringer's instead of salt solution. Therefore, in the perfusion or washing of delicate anatomical structures, physiologically balanced solutions must always be used. But even such a solution is harmful if it has not the same osmotic tension as the tissues. It is very probable that the osmotic tension of Locke's solution is not exactly suited to the cat's kidney. Rathery[23] has shown that slight variations of the cryoscopic point of the solution in which a fragment of the kidney is preserved is able to modify in a large measure the morphology of the cells. For the rabbit's kidney, the best solution has a cryoscopic point of —0.78 C. All other solutions are nephrolytic. If, for instance, a solution which freezes at —0.90 C. is used, the cells are found retracted and as having expelled into the lumen of the tubules a great many of their nuclear granulations. In order to prevent the osmotic disturbances that Locke's solution probably produces in some measure on the cat's kidney, it would be necessary to determine accurately the cryoscopic point of the solution isotonic for the cat's kidney, and to use then a balanced solution of this same tension.

Nevertheless, even an iso-osmotic, physiologically balanced solution would not be able to keep the kidney in its normal condition. Salkowski[24] has shown that organs kept at body temperature under conditions which prevent bacterial growth undergo self-digestion. The autolysis of liver and kidneys, etc., is due to proteolytic enzymes which are contained in the cells and come into play as soon as the circulation is stopped. Opie[25] succeeded in isolating two proteolytic

[20] Howell, *Amer. Jour. of Physiol.*, 1898, ii, 57.

[21] Cushing, *ibid.*, 1901–02, vi, 77.

[22] Flexner and Jobling, verbal communication.

[23] Rathery, Le tube contourné du rein, étude histologique, anatomopathologique experimentale, Thèse de Paris, 1905.

[24] Salkowski, *Zeit. f. klin. Med.*, 1890, Suppl., xvii, 77.

[25] Opie, *Jour. of Exper. Med.*, 1907, ix, 207.

ferments from the leucocytes and the lymphocytes, leucoprotease and lymphoprotease, and discovered that these enzymes are held in check by an antibody present in the serum. He was able also to isolate this antibody and to show that in a relatively small quantity it can neutralize the action of the autolytic ferments.

The researches of Opie explain why a mineral solution is not able to preserve tissues in normal condition, since autolysis soon occurs. To hold in check the activity of the proteolytic ferments set free by the suppression of the circulation, it is necessary to use a fluid containing in some proportion the antibody of the serum. The simplest method would be to use normal serum for perfusing the kidney. Another method consists of cooling immediately the organ to + 1° C. a temperature at which the enzymotic activity is almost completely suppressed.

In the first experiments, the kidneys were thoroughly perfused with Locke's solution. In the last ones, the perfusion was very incomplete, a great deal of blood being still mixed with Locke's solution. This change was made with a view of leaving in the vessels of the kidney a little normal serum and contained anti-enzymotic bodies. But the amount was probably insufficient to prevent autolysis.

After the circulation has been reëstablished, the kidneys are not, however, in normal condition, and their cells are still exposed to many causes of injury.

The blood pressure of the host may differ from that to which the kidneys were accustomed. Possibly, the serum of the host is injurious, in some cases, to the new organs. Generally, however, the serum of an animal has no cytolytic action on the cells of another animal of the same species. Nevertheless, Ehrlich has shown that isocytolysins exist. Consequently, it may happen that the cells of the transplanted kidneys are injured by the serum of the host, even of the same species. This is probably an exceptional complication.

The isolation of the nervous apparatus of the kidneys from the central nervous system may be the cause, direct or indirect, of anatomical lesions. The denervation appeared as a grave objection to the possible efficiency of the transplanted kidneys. There is no

physiological evidence of the existence of secretory renal nerves. However, denervation of the kidney produces, according to Bindo de Vecchi,[26] degenerative lesions of the epithelial cells of the tubules. Marked disorders of the renal functions followed the section of the nerves in the experiments of Krimer, Brachet, Muller and Peipers. The urine, very abundant and diluted, contained albumin and even blood corpuscles. It is, of course, impossible to ascertain whether these changes are due to the section of the hypothetical secretory nerves or merely of the vaso-motor nerves or, perhaps, to other secondary causes. But, even if the denervation alone be able to bring about these results, it need not be considered as especially dangerous. An animal can live in good health after section of the renal nerves. Floresco[27] dissected and cut out the nerves of the left kidney of a dog. Fifteen days afterwards, he resected the right kidney. The animal remained in good health. Last year, an experiment still more complete and conclusive was performed by me at the Rockefeller Institute. In the same operation, the right kidney of a bitch was extirpated and the left kidney isolated and left united to the body only by its ureter and vessels which were dissected as closely as possible. Practically all the nerves were severed. Nevertheless, five days after the operation, the amount of urine voided was 130 cubic centimeters. There was no albumin and the animal was in a normal condition. After one month the animal was found secreting from 90 cubic centimeters to 124 cubic centimeters of urine with high specific gravity and without albumin in the twenty-four hours. Eight months after the operation, he was in excellent health. This demonstrates that the denervation of the kidneys is of little importance for the general health of the dog. It is probable, however, that the kidneys, being deprived of the powerful protection of their nervous system, are more sensitive to pathological insults than the normal kidneys are. Had the animal been allowed to live as street dogs do instead of being kept quietly in a cage at an even temperature, with good food and without muscular exertion, perhaps pathological changes would have ensued.

[26] de Vecchi, *Arch. di farmacol. sperimentale e scienze affini*, 1906, v, 433, 479.
[27] Floresco, *loc. cit.*

CONCLUSION.

Among so many etiological factors, it is impossible to discriminate which are responsible for the complications which took place in our experiments. An attempt to explain the occurrence of nephritis, œdema or calcification of the arterial system, for instance, will not be made, but the technique of the operations will be modified in order to suppress as much as possible the causes which may originate these secondary changes. The purpose of this article was not to analyze minutely the physiological or pathological character of the functions of transplanted kidneys, but merely to ascertain whether these functions are efficiently reëstablished.

It is to be concluded that an animal which has undergone a double nephrectomy and the grafting of both kidneys from another animal can secrete almost normal urine with his new organs, and live in good health at least for a few weeks. This demonstrates that it is possible to reëstablish efficiently the functions of transplanted kidneys.

EXPLANATION OF PLATES.

PLATE XI.

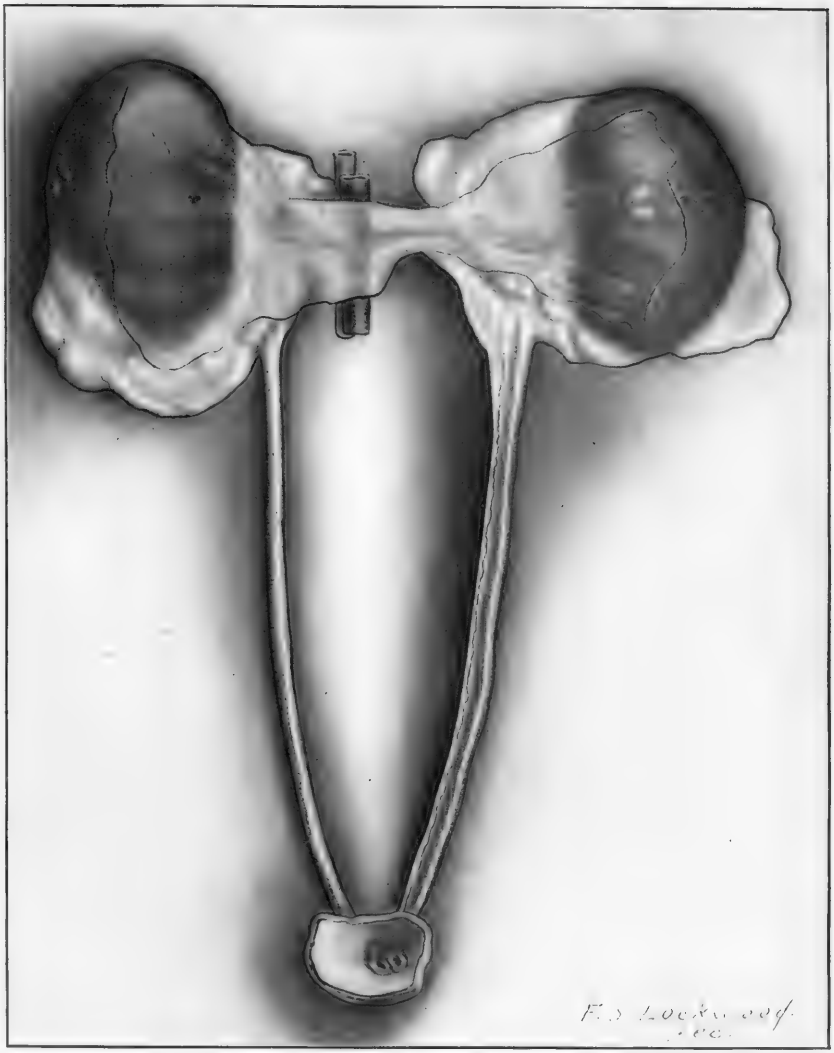
Anatomical specimen extirpated from the first animal and ready for transplantation to the second animal (host).

PLATE XII.

The host ready for the reception of the anatomical specimen of Plate I.

PLATE XIII.

Specimen taken from Cat 7, showing the transplanted kidneys, and cicatrized vascular anastomoses and flap of bladder.



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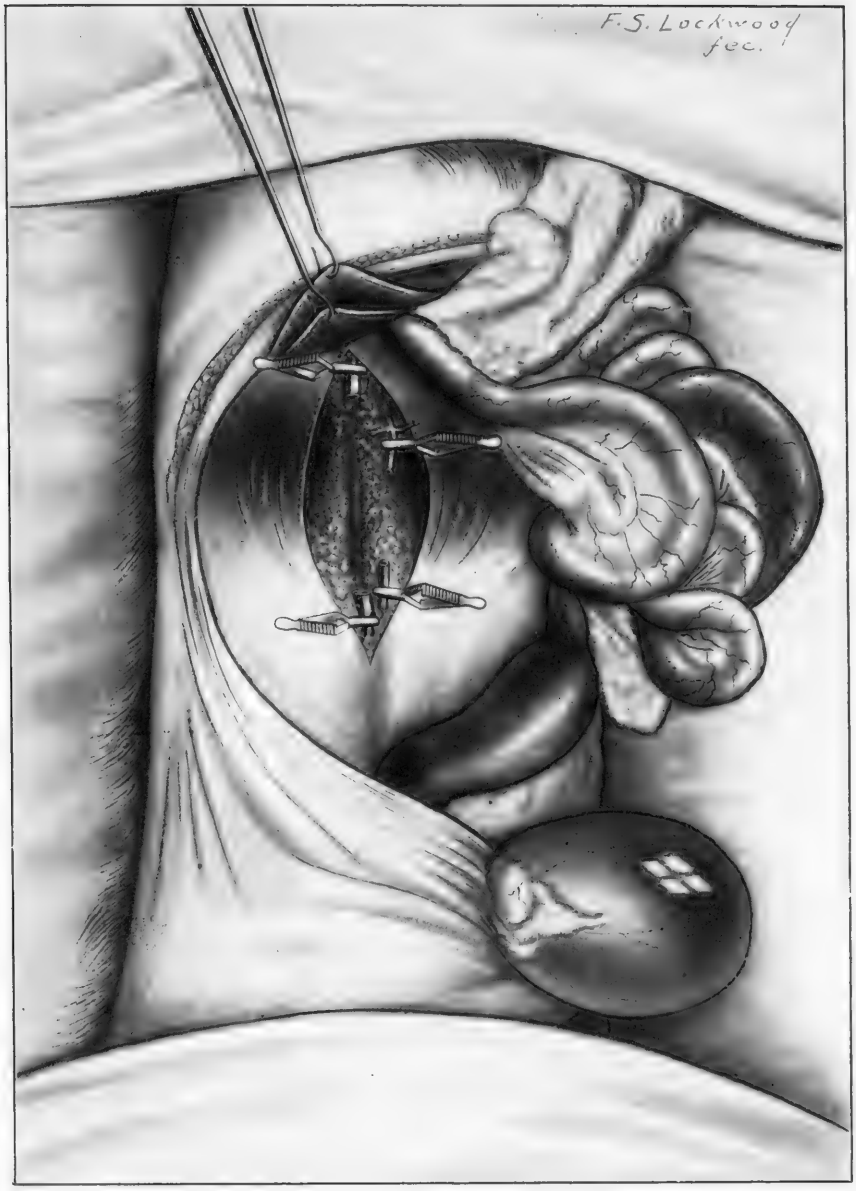
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SERUM TREATMENT OF EPIDEMIC CEREBRO-SPINAL MENINGITIS.*

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INTRODUCTION.

During the prevalence of the epidemics of cerebro-spinal meningitis in America and Europe from 1904 to 1907 *Diplococcus intracellularis,* discovered by Weichselbaum in 1887, was established finally as the cause of epidemic meningitis. In the course of the studies of this microorganism carried out by one of us (Flexner[1]), as one of a commission appointed by the Department of Health of the City of New York to investigate epidemic meningitis, an attempt was made to modify favorably the course of experimental infections with the diplococcus in animals by antisera prepared in several kinds of small animals from *Diplococcus intracellularies.* The ultimate purpose of these experiments was the employment of an antidiplococcus serum in the human infection once it was shown that it could be effective in the experimental infections. Flexner's first reports established that guinea pigs and monkeys, in which the conditions of the infection could be controlled, can be saved from otherwise fatal effects of the diplococcus by the use of the antiserum. Up to the time the first report was published a sufficient opportunity to test an antiserum in human beings had not appeared. Since then a diplococcus antiserum prepared by us in the horse has been tested upon several series of cases of epidemic meningitis, occurring in New York, Philadelphia, Cleveland, Castalia and Akron, Ohio, Edinburgh, Scotland, and Belfast, Ireland. The report which follows deals exclusively with the results of the use of the antiserum in human beings affected with epidemic meningitis.

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[1] *Jour. of the Amer. Med. Assoc.,* 1906, xlvii, 560. *The Jour. of Exper. Med.,* 1907, ix, 168.

The tests of the antiserum upon which this report rests could not have been carried out without the cordial coöperation of a considerable number of physicians who showed great interest in the undertaking. It will not be possible for us to thank personally, or even by name, all those participating in the tests and we will have, therefore, to content ourselves with the mention of those physicians who were very active in carrying them out. To Dr. L. W. Ladd, of Cleveland, who carried the antiserum to Castalia and Akron, Ohio, and who was the first to employ it systematically in a series of cases of meningitis, we feel an especial and deep obligation, on account of his early interest and the difficulties which he encountered in Castalia in following the cases which arose at widely separated points in a sparsely-settled country district. We are also grateful to Dr. Crile, of Cleveland, who brought to our attention the epidemic at Castalia and selected Dr. Ladd to administer the serum, to the physicians of the City Hospital of Akron, for their interest in the subject and the full reports which they supplied, to Dr. W. T. Longcope of the Pennsylvania Hospital, and Dr. B. F. Royer of the Municipal Hospital, Philadelphia, to Dr. L. Emmett Holt, of the Babies Hospital, and Dr. Strain of St. Vincent's Hospital, New York City, to Dr. Harvey W. Cushing, of Baltimore, Dr. Claude B. Ker, of Edinburgh, and Dr. A. Gardner Robb, of Belfast, and to the attending staffs at the several hospitals who permitted the trials to be made on their patients.

There will now follow the records of the cases of epidemic meningitis treated with the serum which are presented, with a few changes, in the precise form in which they came to us. The only alterations made in the reports consist of abbreviations of the hospital records where certain details could be omitted with a view of saving space and time, and the addition, at the end of each case, of a brief discussion, the purpose of which is to re-present the salient features with especial reference to the influence on the course of the disease exercised by the lumbar punctures and serum injections.

We regret that in some instances we have not yet received the full reports of cases treated with the antiserum, and are, therefore, restricted to the use of brief statements given in letters to one of us

(Flexner). These omissions are at present unavoidable and have been brought about by the great distances from New York at which antiserum is being tried, or by other circumstances which have temporarily caused withholding of the records. We believe that should the epidemic in America suffer a recrudescence the antiserum will receive a larger and more searching test; and, in any case, since the disease is still appearing sporadically over a wide territory in America and threatens to reappear in force in Great Britain, we expect to be able to publish a second and more complete report on the serum treatment of epidemic meningitis at no very distant date.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT AKRON, OHIO.

The epidemic at Akron began in April, 1907, and embraced about twenty cases of meningitis. We are greatly indebted to Dr. W. S. Chase for many of the facts on which are based our consideration of this epidemic. Between May 9 and June 16 there were reported to the health officer, as having been treated outside the hospital, nine cases of meningitis of which eight died and one recovered. Dr. Chase states that the patient who recovered presented atypical symptoms and no bacteriological examination of the spinal fluid was made. None of these cases received the antiserum.

Eleven cases of epidemic meningitis, established as such by symptoms and by bacteriological examination, were treated in the City Hospital with the antiserum. Eight of the cases recovered and three died. The period of first injection of the antiserum, the total amount of antiserum injected, and the mode of termination of the disease in the eleven patients are tabulated below.

Case	I,	1st injection	5th day;	total serum injection		82.5 c.c.; recovery by lysis.
"	II.	"	30th hour;	"	"	10.0 c.c.; died.
"	III.	"	12th day;	"	"	20.0 c.c.; recovery by lysis.
"	IV.	"	2d day;	"	"	43.5 c.c.; recovery by lysis.
"	V.	"	7th day;	"	"	25.0 c.c.; recovery by crisis
"	VI.	"	2d day;	"	"	22.5 c.c.; died.
"	VIII.	"	1st day;	"	"	35.0 c.c.; recovery by crisis
"	VII.	"	1st day;	"	"	15.0 c.c.; died
"	IX.	"	2d day;	"	"	105.0 c.c.; recovery by lysis
"	X.	"	1st day;	"	"	25.0 c.c.; recovery by crisis
"	XI.	"	2d day;	"	"	22.5 c.c.; recovery by crisis

Two of the fatal cases were of the fulminating type, and one (Case VI) was injected first with the serum at the end of the second day and again on the fourth day of the illness and died three hours after the second injection. The fulminant cases died six hours and ten hours respectively after the first serum injection.

The total number of cases treated with the antiserum at Akron being eleven it is obvious that little value can be attached to the results stated in percentages. However, the following comparison may be made.

Nine cases of meningitis untreated with the antiserum: Eight or 89 per cent. died and one or 11 per cent. recovered.

Eleven cases treated with the antiserum: Eight or 72 per cent. recovered and three or 27.3 per cent. died.

Eliminating from the calculations the fulminating cases as being beyond reach of treatment, the figures obtained are:

Nine cases treated with the antiserum: Eight or 89 per cent. recovered and one or 11 per cent. died.

City Hospital, Akron, Ohio, Service of Dr. W. S. Chase.

CASE I. V. H. White female, aged 12 years. School girl.

Present Illness.—At 1 a. m. April 28, patient complained of pain in legs and stomach; at 4 a. m. became nauseated and vomited. She remained in bed during the greater part of the day and vomited frequently. Late in afternoon arose and walked out for 15 minutes. At 7 p. m. she became unconscious and voided urine involuntarily. She was restless and noisy until 2 a. m., April 30, when she slept for 2 hours; then the restlessness returned. Rigidity of neck and slight retraction of the head were first noticed on the 30th instant. Admitted to hospital that day.

Physical Examination.—Patient unconscious and restless and screaming, neck rigid, head retracted, pupils equal and react to light, patellar and abdominal reflexes absent, Kernig's sign marked, Babinski not present, herpes on lips. Temperature 99.6°, pulse 100, respiration 24.

May 1. Totally unconscious, restless and noisy; temperature ranged from 99.6° to 102.2°; pulse from 92 to 160.

May 2, 10 a. m. Lumbar puncture, 15 c.c. of opalescent fluid withdrawn. Microscopical examination showed Gram-negative diplococci within and outside of pus cells. Temperature from 99.6° to 102°.

May 3. Condition unchanged.

May 4, 6 a. m. Temperature 101.8°.—8.30 a. m. 10 c.c. opalescent fluid withdrawn by lumbar puncture and *10 c.c. antimeningitis serum injected.*—10 a. m. Temperature 100°.—10.40 a. m. Convulsion involving face, eyes and left hand lasting two minutes.—11.21 a. m. Second convulsion, which continued until relieved with chloroform.—1.50 p. m. Continuous nystagmus of both eyes; twitch-

ing of arms and upper lip; reflexes of extremities and cornea absent.—3 p. m. Convulsions continue except when controlled with chloroform. Kernig's sign more marked than before; rigidity of neck increased; convulsions controlled with chloroform until 5.30 p. m., nystagmus continuous between convulsions.—6 p. m. Temperature 102.2°.—8 p. m. 99.8°.—8.45 p. m. Conscious.—9.30 p. m. Convulsion controlled with chloroform.—12 midnight. Temperature 98.2°.

May 5. Temperature did not rise to 100° until 9 p. m. Patient slept from 12.30 a. m. to 3 a. m. Still some twitching of mouth and left eye. At 4 a. m. took water on being aroused. At 6 a. m. complains of thirst and asks constantly for water and milk.—7 p. m. The patient has taken freely of milk and water during the day and cries for food. She has rested well until midnight. Temperature below 100° until 9 p. m., when it was 100°, and at midnight 101.4°.

May 6. Rested fairly well; condition improved; took considerable nourishment.

May 7 and 8. No essential change.

May 9. Restless after midnight, complains bitterly of frontal headache; temperature rose at 12 m. to 102.8°, and at 3 p. m. to 103.4°.—9 p. m. Temperature 103.8°; lumbar puncture under chloroform anæsthesia, 30 c.c. opalescent fluid withdrawn and *7.5 c.c. antiserum injected.*—10.30 p. m. Temperature 101.8°.—12 midnight. 101.6°.

May 10, 2 a. m. Slight twitching of hands; vomited. Rested well and took nourishment during the day; temperature normal to subnormal.

May 11. Temperature remained below 100°; patient rested well.

May 12, 3 a. m. Temperature 100°; very restless and noisy. Temperature rose during the day.—9 a. m. 102.8°.—12 m. 103.6°.—9 p. m. 104.2°. Withdrew, under chloroform anæsthesia, 60 c.c. opalescent spinal fluid, in which diplococci were not found on microscopical examination, and injected *7.5 c.c. of the antiserum.*—12 midnight. Temperature 102°.

May 13. Patient had a good day and the temperature was 100° at 6 a. m., normal at noon and 97° at midnight.

May 15. Complains of bad headache; temperature rose in afternoon to 104° (3 p. m.).—9 p. m. Temperature 103.2°, lumbar puncture yielding 22 c.c. opalescent fluid free of diplococci.—12 m. Temperature 103.8°.

May 16. Temperature remained above 100° until 9 p. m. Vomited several times; very restless. The condition fluctuated, but remained essentially unchanged until May 25. The rigidity of the neck continued and Kernig's sign was still present and the reflexes had returned in some degree. Pain was still complained of in the head and other parts. The temperature fluctuated between 99° and 102.5°. At 11 a. m. 90 c.c. of less opalescent fluid were withdrawn by lumbar puncture and *20 c.c. of antiserum injected.* The spinal fluid contained leucocytes but no diplococci. At 8 p. m. marked urticaria developed. The patient vomited and was restless and noisy during the afternoon. The temperature fell and reached normal at 12 p. m. the next day. The temperature remained at normal or a little below for four days, then rose to 101.8°, and fell in a few hours. The general condition was better.

June 1. Herpes appearing on lips; neck rigidity lessened; Kernig's sign still marked. Temperature fluctuated between 99.4° and 101°.

June 3. Restless, noisy, complains of pain. Temperature rose suddenly at

9 p. m. to 103°.—11 p. m. Lumbar puncture under chloroform anæsthesia. 75 c.c. of fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid showed on microscopical examination a few diplococci and a very small number of leucocytes. Temperature at midnight 100.4°. The temperature was normal June 4, and did not rise above again. The note on June 9 reads: "Patient's condition greatly improved; she is rational and quiet all the time, the reflexes are normal, Kernig's sign is absent, the rigidity of the neck has disappeared, there is complete absence of all pain, and all the functions appear to be normal. The patient states that she feels well."

June 10 to 14. The note states that the temperature has remained practically at normal; sleep is good, but there is some complaint during the day of pain in various parts; the reflexes are normal and Kernig's sign is absent, but there is involuntary movements of bowels and bladder.

June 15. The note states that the patient still complains of pain and is very restless and noisy. The temperature is about normal. 2 p. m. Headache and vomiting. At 3 p. m. lumbar puncture was performed and 75 c.c. of spinal fluid were withdrawn. Microscopical examination showed "many extracellular diplococci and no leucocytes." *22.5 c.c. of antiserum injected.* The next notes are given entire.

"From June 17 to 25, inclusive, the temperature ranged from normal to 101.6°. The latter temperature was reached on the 18th instant; after that it did not again reach 100°. Appetite was good. Pain in the ear and abdomen complained of responded to palliative treatment. Involuntary micturition and bowel movements continued. On the 25th the patient sat up in bed without discomfort."

"Beginning June 26 (58th day of disease) the temperature remained normal until the day of discharge (96th day of disease). After June 28 the involuntary movements ceased. . . . On 96th day the patient was examined and found well; discharged. She reports to hospital twice weekly, and she is in excellent condition."

Discussion.—This case is one of severe and protracted epidemic meningitis. The diagnosis is clearly established by the symptoms and the bacteriological examination. The special interest which the case has for us is involved in the question whether its course was essentially influenced by the several injections of antimeningitis serum. It does not seem possible to give a definite and outright answer to this question. On the other hand the following points appear to be clear: The first injection of serum (on May 4) was followed by severe convulsions enduring during a large part of the day and requiring to be relieved by chloroform. The convulsions did not re-appear spontaneously and were not excited by subsequent injections of larger amounts of the serum. It is probable, therefore, that the association was accidental. The serum injections generally were followed by a fall in the temperature which reached

or approached the normal and remained at these levels for several days. The temperature and symptoms were subject to much fluctuation, but when the former rose approximately to 104° and the latter became severe, lumbar puncture and serum injection were followed by a tolerably prompt improvement in the patient's condition. On one occasion (May 15), lumbar puncture was performed and no serum injection made and it is noteworthy that the temperature did not fall as in the instance in which the serum injection followed the puncture. The condition of the patient becoming unsatisfactory on May 25 a puncture and serum injection being carried out the temperature fell promptly, remained at or about normal for several days, and the patient's general condition was described as improved. These different results may mean nothing actually, but we put them together since it is desirable to secure light on the independent effects of the puncture alone and the puncture plus the serum injections. Note should be taken of the occurrence of urticaria following the serum injection of May 25. The clearing up of the cerebro-spinal fluid, following the puncture and serum injections, and the disappearance from it of many leucocytes and all *demonstrable* diplococci before the subsidence of the symptoms is shown to be possible. The final history of the case indicates that there may still remain an active focus in the membranes from which a fresh invasion of diplococcus into the spinal fluid may take place, with which is associated a reappearance of certain symptoms and a sudden rise in temperature. Such a relapse would seem to have occurred on June 3 and not to have been attended by a rich outpouring of leucocytes into the cerebro-spinal fluid. If this observation is admitted it is at least worth noting that the condition was quickly controlled by the puncture and serum injection although it is not established that these means were the sole or chief causes of the abrupt termination of the relapse. Only an accurate and painstaking clinical and bacteriological study of protracted and relapsing cases of epidemic meningitis will suffice to determine the manner in which, and the rapidity with which the body's forces unaided deal with the diplococcus. Total amount of antiserum injected, 82.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. George Rankin.

Case II. H. R. White male, aged 17 years. Rubber worker.

Present Illness.—Admitted May 9, 1907, 12 m. The day before admission the patient was at his work, but in the evening he complained of headache and feeling ill, and he went early to bed. At 4 a. m. his family was aroused by his falling out of bed. He was picked up in an unconscious state. He was seen at 9 a. m. by a physician who found him unconscious, restless, throwing himself about, and with a temperature of 104° F. Still unconscious and tossing about on admission to hospital at 12 o'clock noon.

Physical Examination.—Face flushed; perspiring freely; pupils moderately dilated, do not react to light; neck rigid and slight retraction of head; abdominal, patellar and plantar reflexes absent; Kernig's sign present.

May 9, 1 p. m. Temperature 101.6°, pulse 90, respiration 40. Lumbar puncture yielded 60 c.c. of opalescent fluid containing pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 101.6°. Vomited several times during the afternoon; pulse could not be counted during afternoon.—6.30 p. m. Temperature 102.8°.—9 p. m. 103°.—12 midnight. 105.4°, respiration 70. At 11 p. m. lumbar punctured and 60 c.c. opalescent fluid withdrawn and *10 c.c. of antiserum injected.* The microscopical examination of the fluid gave the same results as the first fluid withdrawn. The pulse continued uncountable and the respiration high. Temperature at 3 a. m. 106.2°, at 5 a. m. 107.2°; died at 5.42 a. m. May 10.

Discussion.—This case is an example of the fulminating type of epidemic meningitis. From the appearance of the marked symptoms and the death, less than 36 hours elapsed. At the time of the second lumbar puncture and the first injection of the serum the patient was in a critical condition and survived these operations only six hours. It is highly improbable that the serum could influence favorably so severe an infection as existed in this case, but it would have been proper to have injected it earlier—immediately after the first lumbar puncture had established the diagnosis—and in much larger quantity. The reason that the serum was not injected earlier is found in the fact that it had first to be brought from Cleveland; and that the dose was small is explained by the date of the serum's employment, for at that time it was being used very cautiously since we had not yet learned that it could be injected in much larger amount with impunity into the inflamed spinal canal of human beings. Total amount of antiserum injected, 10 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE III. B. S. White male, aged 19 years. Rubber worker.

Present Illness.—Until May 11, 1907, the patient was well. On that day he felt badly, went to bed early and was said to have had a chill during the night. No further history of illness until May 13, when a physician saw the patient who was feverish, restless and complained of pain in head and neck. That night he became unconscious; admitted to hospital next morning (May 14). He was, at that time, unconscious and irrational and very restless, tossing and talking continuously. "Temperature 99°, pulseless at wrist, respiration 32 and shallow, cyanosed."

Physical Examination.—Unconscious and greatly cyanosed young man. Heart beats 56 per minute. Neck somewhat rigid and painful on being moved. Reflexes diminished.

May 14. Temperature varied from 99° to 101.6°. Under stimulants, pulse improved; at 12 p. m. 104, at 12 a. m. 88.

May 15 and 16. Condition essentially unchanged.

May 16. Reflexes absent from extremities and abdomen; neck rigidity marked; Kernig's sign present. Lumbar puncture unsuccessful. Restless and talking.

May 23. Note states: "Patient semi-conscious part and conscious other part of the time. The reflexes, except the plantar reflex, are absent. Neck rigid and painful on being moved. Temperature fluctuated from 99° to 102.4°. Slept and rested well and took considerable nourishment."

May 24. Temperature rose to 103.4°; otherwise no change.

May 26. No marked change. Kernig's sign present; reflexes absent. Temperature at 12 p. m. 103.6°. Lumbar puncture performed under local anæsthesia and 60 c.c. opalescent fluid obtained and *20 c.c. antiserum injected.* The examination of the spinal fluid showed pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 103.2°.—6 p. m. 102.8°.—9 p. m. 102°.—12 a. m. 99.8°. Patient rested well, perspired freely and took considerable nourishment during the night.

May 27. Temperature ranged from 98° to 99.2°. The note states: "Mental condition improved; reflexes not changed; slept well and took considerable nourishment."

May 28. Temperature ranged from 97° to 97.8°. The note states: "Reflexes are now normal; the mental condition is good, but the neck is still somewhat rigid and slightly painful on being moved."

The next note states that from May 29 to June 6, the date of discharge from the hospital, the patient's condition continued to improve and there was complete recovery.

Discussion.—This case is an example of epidemic meningitis with severe onset and gradual subsidence of symptoms by lysis. The indications are that the patient's recovery was reasonably assured before the successful lumbar puncture and injection of the serum on the twelfth to the fourteenth day of the disease. Until the day of injection the temperature had not remained continuously, during any

one day, below 101°. Within twelve hours of the injection the temperature fell below 100° and did not again rise to that point, tending rather to remain somewhat subnormal. Whether this is more than a coincidence cannot be decided now. The quick return of the reflexes following the puncture and serum injection, and the acceleration of the rate of improvement in the patient's mental and general condition, may also be merely co-incidental, but they were sufficiently great to be regarded as noteworthy. Total amount of antiserum injected, 20 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. L. R. C. Eberhard.

CASE IV. B. K. Female, aged 15 years. Factory worker.

Present Illness.—Patient felt well and worked until May 14, 1907. Awoke 6 a. m. May 15 feeling ill and complaining of headache; ate little breakfast; returned to bed, vomited and became unconscious during the day. Physician called; found temperature 101.4°; patient restless and irritable and crying out with pain on being touched. Admitted to hospital May 16. She was unconscious and tossing about. The abdominal reflex was absent and the patellar and plantar reflexes greatly diminished. The neck was somewhat rigid and attempts to move it were very painful.

May 16, 9 a. m. Temperature 103.8°, pulse 84, respiration 34.—10.30 a. m. Lumbar puncture under chloroform anæsthesia; 45 c.c opalescent fluid containing pus cells and extra- and intracellular diplococci withdrawn. *15 c.c. of antimeningitis serum injected.*—12 p. m. Temperature 102°. It ranged during the rest of the day from 101° to 102.6°.

May 17, 3 a. m. Temperature 99.6°; 10 a. m. 100.8°; 5 p. m. 102.2°; 12 a. m. 102.6°. The note states: "Patient slept part of the night, but is restless at times. Knee, plantar and abdominal reflexes are present. There is marked Kernig's sign and no Babinski reflex; no ankle clonus. Restless and noisy from 9 p. m. to midnight."

May 19, 8 a. m. Unconscious and irrational except when spoken to. Herpes on lips. Internal squint of right and left eyes.—7.30 p. m. Lumbar puncture under chloroform anæsthesia; 45 c.c. of opalescent fluid withdrawn and *5 c.c. antiserum injected.* Slept from 11 p. m. to 5 a. m.

May 21. Condition has remained essentially unchanged. Temperature, 9 a. m., 103.2°.—9.30 a. m. Lumbar puncture: 3.5 c.c. fluid withdrawn and *3.5 c.c. serum injected.* Spinal fluid shows diplococci.—7 p. m. Condition unchanged.

May 22. Patient irrational and complains more than previously on being moved. She seems not to have control of the arms, although she moves hands, fingers and legs.

May 25. Condition has not changed materially. 9.30 a. m. Lumbar puncture under chloroform; 75 c.c. of opalescent fluid removed; *20 c.c. of antiserum injected.* Temperature during the day ranged from 99.6° to 102.8°.

May 26. Temperature ranged from 99.8° to 102°.

May 27. 99° to 102.6°. The note at 6 p. m. states that all reflexes are present, that the left arm cannot be used and the left trapezius is contracted, drawing the

head to the left side. Patient is conscious and rational and rested well. Kernig's sign is present.

May 27. Urticaria has appeared on knees and elbows. The temperature has remained below 100° since 3 a. m.

May 29. Temperature below 100°.

June 1. The temperature has remained below 100° except for one or two brief intervals, when it reached that height. Patient is conscious and rational. All reflexes except the abdominal reflex are present, the neck is less rigid and can be moved voluntarily as can the arms and legs. Kernig's sign still present.

June 5. Temperature has remained below 100° (all temperatures until to-day taken per rectum). Axillary temperature normal.

June 9. Patient improved in every way. Reflexes normal. Kernig's sign absent. Rigidity of neck gone. Has use of all extremities and functions appear to be normal. Urticaria appeared over entire body.

June 10. The final note states that temperature and pulse remained normal and the patient was discharged well on June 27, the forty-first day after admission.

Discussion.—This case is an example of epidemic meningitis with severe onset, moderately prolonged illness, subsidence of symptoms by lysis and complete recovery. The patient was admitted to the hospital about 24 hours after the appearance of the first severe symptoms, and the diagnosis was established by lumbar puncture and a first dose of antiserum administered within the first thirty hours of the disease. No marked or permanent influence on the course of the disease, as far as can be determined, was produced by the puncture and serum, and subsequently three additional injections of serum (following withdrawal of fluid) were made into the spinal canal. On May 25, or approximately the tenth day of illness, an injection of 20 c.c. of serum was given (the two previous injections were of 3.5 c.c. and 5 c.c. respectively). On May 26, the note states that the reflexes, which had previously been absent, had returned, but the same note records the involvement of the trapezius muscle in the rigidity of the neck. May 27 the temperature remained persistently below 100°, and from that date on no rise of temperature above 100° (rectal measurements) was recorded. Urticaria appeared on that day. The condition of the patient improved more or less in the next days and on June 1, the symptoms had considerably abated, voluntary muscular movements had returned and the neck was less stiff. The disappearance of Kernig's sign and the general functional restoration of the body

are noted on June 9 when the patient was regarded as convalescent. It is not possible to assign the specific influence, if any was exerted, of the serum on the progress and final result in this case. Total amount of antiserum injected, 43.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE V. F. N. White male, aged 32 years. Laborer.

Present Illness.—The patient had been well, except for coryza, until May 12, when he felt ill and suffered from pain in the muscles and head. May 13 consulted physician who said he had fever and who gave him a purgative and sent him to bed. Did not go to work, felt chilly, vomited, complained of pain in head and neck. Next day no better, but he went to his work and remained until noon. He was obliged to return to bed, where he remained until May 16, when his mental condition becoming alarming he was taken to hospital. Patient became unconscious in the ambulance.

Physical Examination.—Unconscious; neck rigid; reflexes absent; Kernig's sign absent; head slightly retracted; pupils somewhat contracted, react sluggishly to light. Temperature, 4 p. m., 104.2°; 12 a. m. 102.2°. Delirious.

May 17. The note states that the patient was restless, noisy and delirious, and Kernig's sign was now present.—3 p. m. Lumbar puncture attempted, but no fluid was obtained. At 2 p. m., out of bed, delirious, put in restraint.

May 18. Neck very rigid, otherwise condition unchanged.

May 21. The condition fluctuated during the past four days; the temperature ranged from 98.6° to 104.4°, pulse up to 130, respiration rapid and irregular, and there were delirium, noisiness and restlessness. The physical signs have not changed; hiccough has appeared.

May 22, 10 a. m. Under chloroform anæsthesia lumbar puncture was made and 90 c.c. of cloudy fluid, which was under considerable pressure, were removed. At the same time *25 c.c. of antiserum were injected.* The spinal fluid showed on microscopical examination pus cells and intra- and extracellular diplococci.—12 m. Temperature 100.8°; pulse 104.—12 a. m. Temperature 102°; pulse 98.

May 23. The note states that the patient rested fairly well after midnight. At 9 a. m. he was conscious and resting quietly and the mental condition was improved. He was free from pain and the reflexes were unchanged. Temperature: 3 a. m. 101.4°, 12 p. m. 100°, 12 p. m. 98°.

May 24. Rested well part of night and day. Conscious and rational. Knee and plantar reflexes present. Kernig's sign still present. Neck still somewhat rigid and painful on being moved. Temperature normal since midnight.

May 25. Temperature normal. The patient has slept well and taken freely of nourishment.

The next note states that from May 25 to June 5 the temperature and pulse were normal; the patient was up and about the ward since June 2, the reflexes were normal, there was no rigidity and no Kernig's sign.

June 6. Patient awoke at 3.30 a. m. complaining of pain in legs and back. He was unable to move his legs and complained of pain when they were moved. Reflexes could not be elicited, but legs were held rigidly. Pain and tactile sensations normal.

June 7. Extremities less painful, but there is marked tenderness over the large nerve trunks.

June 8. Functions of lower limbs gradually returning.

June 12. The patient is improving, the neuritis is diminishing and the extremities can be used.

June 15. Patient discharged cured.

August 2. Patient has reported to the hospital. He is in perfect health.

Discussion.—The onset of the symptoms of meningitis was in this case gradual and extended over 3 days. The symptoms became severe on May 16 and the patient's condition was bad until May 23 when it suddenly and critically changed for the better. From May 23 on the patient's condition continued to improve, with the exception of the discomfort caused by the neuritis which appeared in the legs on June 6 and quickly subsided, until June 15 when he was discharged "cured" from the hospital. The meningeal and mental symptoms and the symptoms of general intoxication were, in this case, profound. On May 22, or approximately the seventh day of the disease, successful lumbar puncture was made and the antiserum injected. Twenty-four hours later the patient's condition was described as improved and the level of the temperature was lower than before. The condition of the reflexes was, however, unchanged. Within forty-eight hours of the puncture and serum injection the patient had become conscious and rational, the reflexes had in part returned, the neck was less rigid, the temperature reached normal, and the patient was resting quietly, sleeping and taking nourishment. No rise in the temperature again occurred. The patient was evidently convalescent. The critical disappearance of the severe symptoms and the lumbar puncture and injection of serum would seem to bear some relation to each other. There was an abrupt transition from a condition of much seriousness, which had endured unchanged for a week, to one of comparative and actual mildness within forty-eight hours of the puncture and injection of the antiserum. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

Case VI. Z. H. White female, aged 17 years. Rubber worker.

Present Illness.—The patient was at work until May 18, when she went home on account of malaise and pain in back and limbs. At 2 a. m. the next morning

she complained of severe headache, at 3 a. m. she became unconscious. A physician was called at 7 a. m. (May 19). He said she had fever. During the day she vomited frequently. Axillary temperature, 3 p. m., 104°. Admitted to hospital 6 p. m.

Physical Examination.—Patient admitted in an unconscious state. She is very restless, tosses and rolls her eyes continuously. Knee and plantar reflexes present; abdominal reflex absent; Kernig's sign present.

May 20. Temperature, 9 p. m., 101.2°. Under chloroform anæsthesia 45 c.c. of spinal fluid were withdrawn by lumbar puncture and *15 c.c. antiserum injected.* The fluid showed pus cells and extra- and intracellular diplococci.—12 p. m. Temperature 99.2°.

May 21. Patient conscious and rational; complains of frontal headache. Pupils equal and react; abdominal reflex absent; patellar diminished, plantar present. Kernig's sign marked. Babinski sign absent; ankle clonus absent. Neck rigid and painful on movement. Temperature has risen above 99°.

May 22. Patient's mental condition less good than yesterday. Herpes appearing on lips. Large hyperæmic areas have appeared on abdomen. Temperature during the day has fallen to 99.8°.—10.30 p. m. Under chloroform anæsthesia withdrew 7.5 c.c. of spinal fluid and injected *7.5 c.c. of the antiserum.*—12 a. m. Temperature 99.2°.—12.30 a. m. Patient had been resting fairly well when the nurse's attention was attracted by gasps and before a physician could reach her side she died. No autopsy was permitted.

Discussion.—The symptoms of meningitis came on quickly and were severe in type and lumbar puncture and a serum injection were made about 45 hours after their first appearance. Following the puncture and serum injection the temperature remained constantly below 100° and the patient regained consciousness. A second puncture and serum injection were made about 48 hours after the first. About two and a half hours after these the patient suddenly died. No autopsy was obtained and the immediate cause of death was not established. Total amount of antiserum injected, 22.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. A. F. Sippy.

Case VII. H. S. White male, aged 14. Schoolboy.

Present History.—Patient complained of headache on May 21. The next day he vomited and suffered pain in back of head. Temperature, 11 a. m. 101°; delirious at 5 p. m. Admitted to hospital at 8 p. m.

Physical Examination.—Temperature 104°, pulse 120, respiration 44. Wildly delirious; pupils dilated, do not respond to light. Reflexes absent; Kernig's sign positive; some rigidity of neck.

May 22, 9 p. m. Under chloroform anæsthesia 75 c.c. of opalescent spinal fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid contained pus cells and extra- and intracellular diplococci. Temperature, 12 a. m., 103°; pulse 100; respiration 30.

May 23. Patient slept until 2 a. m., after which time he was restless. Temperature: 3 a. m. 102.4°, 9 a. m. 99° 9 p. m. 100° (by rectum).

May 24. Temperature: 12 p. m. 100.6°, 9 p. m. 99.8°. Pulse 60 to 78. Vomited twice; rested fairly well.

May 25. Patient slept fairly well after-part of night; complains of headache. Neck rigid and painful on motion; pupils react to light. Patient conscious and rational. Temperature: 1 a. m. 103°, 9 a. m. 102°. At 10 a. m. 75 c.c. of opalescent spinal fluid withdrawn and *20 c.c. antiserum injected.* Microscopical examination of the spinal fluid showed leucocytes and diplococci. Temperature: 12 p. m. 100°, 6 p. m. 99°, and 12 p. m. 98.6° (mouth).

May 26. Temperature normal. Patient slept well after-part of night; conscious and rational; rests quietly; all reflexes present; neck less rigid than earlier in attack.

May 27 to June 1. The temperature has remained normal and his condition satisfactory. Note on June 2 states that the patient is apparently well, the reflexes are normal and the rigidity of the neck has gone.

The final note states that from June 3 to June 8, the day of discharge from hospital, the temperature remained normal and recovery was perfect.

Discussion.—The patient was brought to the hospital during the first day of illness and the diagnosis of meningitis was established by lumbar puncture and the first dose of serum administered in little more than 24 hours after the illness began. The subsequent course of the infection was mild. On the fourth day of illness (May 25) the temperature having risen to 103° F. a second puncture and serum injection were made. Less than twenty-four hours later the temperature had fallen to normal and the general condition of the patient had improved. There was no subsequent rise in the temperature, all the symptoms rapidly subsided, and recovery was complete. Total amount of antiserum injected, 35 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

Case VIII. R. M. White male, aged 17 years. Electrician with rubber company.

Present Illness.—On May 24 left his work at noon on account of feeling ill. Went home, refused supper, vomited during the evening, and went to bed early on account of headache. Restless during the night. Headache more severe next morning; vomited again, and became unconscious about 10 a. m. Admitted to hospital 1 p. m.

Physical Examination.—Well-developed, muscular man. Pupils slightly contracted; equal; react to light. Neck somewhat rigid and painful on being moved. Knee reflex exaggerated, plantar reflex present, abdominal reflex absent. Kernig's sign present. The patient is very violent and tosses on the bed.

May 25, 2 p. m. Temperature 105.2°, pulse 142, respiration 20. Under chloroform anæsthesia 15 c.c. of spinal fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid showed leucocytes and extra- and intracellular diplococci.—6 p. m. Temperature 104.8°, pulse 134.—9 p. m. Temperature 102.4°, pulse 130, respiration 20, cyanosed. Aromatic spirits of ammonia and ether administered hypodermically. Respiration ceased; artificial respiration and oxygen inhalation; respiration continued from 6 to 8 per minute; death at 12.30 a. m.

Discussion.—The preceding case is an example of the fulminating type of epidemic meningitis in which from the first appearance of severe symptoms and death about 24 hours elapsed. Lumbar puncture and serum injection were made about 10 hours before death and were without appreciable effect on the course and termination of the infection. Total amount of antiserum injected, 15 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. George Rankin.

CASE IX. J. A. S. White male, aged 24 years. Mail carrier.

Present Illness.—Until two days before admission to hospital patient has been in good health. He ascribed headache and malaise to exposure to hot sun. June 23 had intense headache, nausea, vomiting and pain in back of neck. About 11.30 p. m. became unconscious. Temperature 103°. Admitted to hospital June 24, 11 a. m.

Physical Examination.—Patient violently delirious; requires to be restrained. Had involuntary bowel and bladder movements. Abdominal and patellar reflexes absent; plantar reflex diminished. Neck rigid; painful on being moved. Kernig's sign present. Pupils equal, moderately dilated, react to light.

June 24, 11 a. m. Temperature 103.8°, pulse 70, respiration 36.—1 p. m. Under local anæsthesia 90 c.c. of spinal fluid removed and *22.5 c.c. antiserum injected.* Pus cells and diplococci chiefly within the cells found on examination of the spinal fluid. Temperature: 3 p. m. 100.8°, 12 p. m. 101.4°.

June 25. Patient slept fairly well under an anodyne during the latter part of the night. Note at 8 a. m.: "Patient quiet and semiconscious, responds when spoken to but mutters unintelligibly when attempting to form sentences. Knee jerk not elicited; plantar reflex diminished; abdominal reflex present for first time since admission. Neck rigidly increased." The temperature fluctuated from 99.6° (6 p. m.) to 101.2°.

June 26. Patient restless during night; conscious and rational part of time; neck held less rigidly. The temperature has risen: at 12 p. m. 102.2°, at 12 a. m. 103° (per rectum).

June 27. Restless; extremities cold. 3 a. m. lumbar puncture under chloroform anæsthesia; 45 c.c. of spinal fluid withdrawn and *22.5 c.c. antiserum injected.* A chill followed lasting 20 minutes. Herpes appearing on lips. The temperature has fluctuated between 102° and 103° all the day.—9 a. m. "The patient in exceptionally cheerful mood, singing and talking, but entirely rational when spoken to."

June 28. Condition essentially unchanged.

June 29. Temperature at a slightly lower level.

June 30. The note reads: "Condition good; perfectly conscious and rational; reflexes all present; Kernig's sign still marked; neck quite rigid and painful on movement; headache; extensive herpes labialis." The temperature fluctuated between 101° and 103.2°.

July 1. Condition about the same.

July 2, 6 p. m. Temperature: 103.4°, 9 p. m. 103.2°. Under chloroform anæsthesia 60 c.c. spinal fluid, containing diplococci, withdrawn and *30 c.c. of antiserum injected.*—12 a. m. Temperature 101.8°.

July 3. The temperature reached 99° and has run at a lower level than previously.

July 4. The temperature (rectal) has remained below 100° all day. Otherwise the condition is not markedly changed.

July 5. The temperature has fluctuated to-day, having reached 102.2° at 9 a. m., but remaining below 100° for the most part.

July 6. The temperature has risen again. At 6 a. m. it was 103.8°; patient did not rest well; the neck rigidity has lessened; mental condition good. Kernig's sign still present.—12 p. m. Temperature 103.4°. At 2 p. m. 45 c.c. of spinal fluid were withdrawn and *30 c.c. of antiserum injected.* Stained specimens of the spinal fluid show very few leucocytes containing diplococci and extracellular diplococci.

July 7. Temperature at a lower level, but general condition not essentially changed.

July 8. Patient rested well. The temperature has been below 100° most of the day.

July 9 and 10. Temperature has not risen and condition of patient improved.

July 11 and 12. The temperature has remained normal.

The final note reads: "July 13 to July 27, the date of his discharge from the hospital, the patient continued to improve. When discharged he complained only of not feeling strong. On August 24, when he reported to the hospital, he was well but had not recovered usual strength. On September 8 he had not yet reported for work."

Discussion.—This case of epidemic meningitis ran a moderately severe and protracted course and the symptoms gradually subsided. The particular influence of the spinal punctures and serum injections cannot be defined. The disease terminated favorably and recovery was complete. The conditions under which the serum was employed were favorable to its action since the first injection was made within 48 hours of the onset of severe symptoms. Four injections of the serum were made without the appearance of unpleasant effects. On the other hand the temperature tended to seek a lower level after the injections. The third injection, on the tenth day of illness, was followed by a fall in the temperature

which persisted for four days. When it again rose to 103.4°, on the fourteenth day a fourth injection of the serum was made after which the temperature fell below 100° and soon reached normal where it remained. Convalescence may be said to have begun on the sixteenth day of the illness. Total amount of antiserum injected, 105 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Kobler and Seiler.

CASE X. E. R. White male, aged 20 years.

Present Illness.—The illness began with headache and nausea about noon. After midday meal the patient went to bed and at 3 p. m. was found unconscious and in a violent state. Admitted to hospital the same day (June 30, 1907) at 9.50 p. m.

Physical Examination.—Unconscious and violently excited man moaning constantly. Knee and plantar reflexes exaggerated; Kernig's sign positive; neck slightly rigid; pupils moderately dilated; react to light.

June 30, 10 p. m. Did lumbar puncture under chloroform anæsthesia and withdrew 75 c.c. of spinal fluid and injected *25 c.c. antiserum.* Microscopical examination of the fluid showed polynuclear leucocytes and many intra- and extracellular diplococci. Temperature: 10 p. m. 100.2°, 12 a. m. 99°.

July 1, 2 a. m. Convulsion lasting 20 minutes. During the day patient vomited several times. The temperature did not rise above 100°.

July 2. The temperature has been below 100° all day and much of the time has been normal. The note reads: "Condition of patient markedly improved; he is conscious and rational, all the reflexes are present, Kernig's sign is less marked than it was, the neck is still stiff and painful, headache still persists, and the mental condition is good. Considerable nourishment was taken during the day."

July 3. The temperature has remained below 100° and much of the time was normal. The general condition is as on yesterday.

July 4. Patient rested well; he complains less of pain. Temperature normal.

July 5. The note reads: "Patient slept well; general condition good; reflexes normal; Kernig's sign and neck rigidity absent; mentality normal."

The patient continued to have normal temperature and was discharged on July 11 "cured," twelve days after having entered hospital.

Discussion.—The onset of the symptoms in this case was abrupt and severe. From the appearance of the premonitory headache and malaise to the lumbar puncture and serum injection hardly more than twelve hours had elapsed. Following upon the puncture and the injection of serum the symptoms abated rapidly and the patient may be said to have been over the disease within forty-eight hours of its onset. No reasonable doubt can exist regarding the diagnosis in view of the symptoms present and the results of

the bacteriological examination. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Theiss and Sippy.

Case XI. G. G. White female, aged 17 years. Schoolgirl.

Present illness.—Twenty-four hours before admission to the hospital she began to complain of malaise and headache. During the night she vomited and the symptoms grew gradually worse until 2.30 p. m. when she became unconscious. Admitted to hospital at 7 p. m. June 9.

Physical Examination.—The patient is unconscious and restless. Abdominal reflex absent; patellar and plantar reflexes are diminished; pupils equal; involuntary bowel movements.

June 10, 9 p. m. Temperature 98.4°, pulse 108, respiration 20. Under chloroform anæsthesia 90 c.c. of opalescent spinal fluid withdrawn and *22.5 c.c. of antiserum injected.* Microscopical examination of the spinal fluid showed many leucocytes and extra- and intracellular diplococci.—12 a. m. Temperature 98.8°, pulse 124, respiration 20.

June 11. Temperature subnormal most of the day. Patient rested fairly well latter part of night. Twitching of face and mouth; head retracted; rational at times.

June 12. Temperature subnormal. Mental condition greatly improved; abdominal and plantar reflexes present; patellar absent. Kernig's sign marked; neck rigid. Herpes appearing on lips. Complains of no pain unless moved.

June 13. Rested well during night. Mental condition quite good; reflexes all present. Kernig's sign and neck rigidity unchanged. In nurse's absence has gotten out of bed and gone to closet. Temperature normal.

June 14. No change.

June 15. Condition good, all the reflexes normal. Almost no rigidity of the neck; Kernig's sign disappearing.

"From this date the patient continued to improve, and she finally made complete recovery. She left the hospital on June 23, thirteen days after admission, and has reported twice since."

Discussion.—The onset in this case was moderately severe, and the diagnosis of epidemic meningitis was established and a serum injection made within forty-eight hours of the appearance of the premonitory symptoms. The course of the disease was relatively mild, and the temperature tended to remain at the normal point or a little below it although the microscopical examination showed the presence in the spinal fluid of large numbers of *Diplococcus intracellularis.* A second lumbar puncture and serum injection were not made as the symptoms abated rapidly and the patient was convalescent on the fifth day of her illness. Total amount of antiserum injected, 22.5 cubic centimeters.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT CASTALIA, OHIO.[2]

The village of Castalia has a population of about 600 persons. Within the village nine cases of meningitis developed. In the outlying country and within three miles of the village, six cases developed. In the country adjacent to the village of Vickery, which is eight miles from Castalia, three cases developed. Thus a total of eighteen recognized cases of meningitis developed in this region between January and April, 1907. The first case appeared near Vickery in January and the remaining seventeen cases appeared between March 1 and April 2. Eleven of the eighteen cases were in adults over 16 years of age, and seven cases were in children between three and six years of age. Of the affected adults nine died and two recovered and of the affected children three died and four recovered.

In the past thirty years sporadic cases of the disease have appeared occasionally, but at long intervals. No case had been recognized previously for five years.

The distance between cases No. 1 and No. 2 was six miles; between No. 2 and No. 3, three miles, between No. 3 and No. 4 one mile. The first three cases occurred in the country. Case No. 4 appeared in Castalia and was followed in rapid succession by eight other cases. Only two of the cases had been in close personal relation with other persons affected.

At the time that Dr. L. W. Ladd brought the antimeningitis serum to Castalia there had been twelve deaths from epidemic meningitis and three cases were convalescent. He employed the serum on three cases as follows:[3]

CASE I. B. K. Female, aged 16 years. Patient of Dr. Storey.

Previously healthy girl. Taken ill suddenly March 30, 1907, with headache, vomiting and flushed face. The temperature was 104° F., pulse 124, respirations 48. Coma supervened within 12 hours. The diagnosis was made day after onset, at which time there were opisthotonos and rigidity of the extremities and petechial eruption of neck, trunk and thighs. The patient remained in coma until 12 a. m. March 31, when she became conscious. April 2 again became unconscious. Dr.

[2] Abstracted from the account of Dr. William Storey, published in the *Ohio State Medical Journal* for June, 1907.

[3] The histories of these three cases are taken from Dr. Storey's report and Dr. Ladd's notes.

Ladd first saw the patient at 7 p. m. April 2. At this time she was semi-conscious, the temperature was 103° F., pulse 120, respirations 48. Lumbar puncture was made, about 45 c.c. of very turbid spinal fluid were obtained and *5 c.c. of the antiserum injected.* The spinal fluid yielded *Diplococcus intracellularis* on coverslips and in cultures on sheep-serum glucose agar. April 3. Temperature 98.5°, pulse 108. Patient quite rational at times, though when not aroused she was delirious.—3 p. m. Temperature 101°; 11 p. m. 103.5°. Second lumbar puncture made, 30 c.c. turbid fluid withdrawn and *10 c.c. of antiserum injected.* The patient remained in a semi-conscious condition for 15 hours when the mental condition cleared; the opisthotonos and rigidity were noted to be much less marked. April 4. Acute bronchitis and broncho-pneumonia developed. The mental condition remained good and the meningeal symptoms were not prominent until April 18, when another puncture was made. About 50 c.c. of turbid fluid were removed and *10 c.c. of antiserum injected.* From this time improvement was gradual but steady. April 24, temperature normal; did not rise again. May 3, slight degree of foot-drop on left side. August 31, recovery complete.

Discussion.—The onset in this case was sharp and the course severe. Withdrawal of spinal fluid and injection of antiserum were made on the third day of illness. A favorable response seemed to follow. Two subsequent lumbar punctures and antiserum injections were made on the fourth and fifteenth day of illness. Following the second injection of serum the mental condition cleared and the rigidity of the body was noted as being diminished. The last injection was succeeded by gradual subsidence of all the symptoms and eventual complete recovery. The meningitis was complicated with an intercurrent broncho-pneumonia. Total amount of serum injected, 25 cubic centimeters.

Case II. F. W. Three years of age. Patient of Dr. Gorsuch.

This child was seen by Dr. Ladd twelve days after the beginning of the symptoms. The onset, consisting of vomiting, convulsions, headache, opisthotonos and great irritability, was sudden. Temperature at onset 104°, pulse 135, respirations 40. The patient had improved somewhat, although there was still great irritability and marked opisthotonos; temperature from 101° to 102.5°. April 3, marked opisthotonos and Kernig's sign; temperature 102°. Lumbar puncture yielded 30 c.c. of moderately turbid fluid. *5 c.c. antiserum injected.* That night, for the first time during the illness, the parents were not aroused. April 4, child less irritable; opisthotonos and rigidity less marked; food taken better. The temperature reached normal and remained so afterwards. April 28, child up and about. August 31, child well.

Discussion.—The abrupt change in the condition in this case was evidently associated with the withdrawal of spinal fluid and the

injection of the antiserum, but the part each played cannot be estimated separately. Total amount of serum injected, 5 cubic centimeters.

CASE III. J. B. Aged 23 years. Patient of Dr. Bowman. Employed on railroad.

Large man of excellent physique. On April 1 went to Freemont, Ohio, to a hotel. Asked to be called early April 2. The door of his room had to be forced open; the patient was found unconscious. He was removed to his home in Vickery and his physician called. On April 4 Dr. Bowman was convinced of the diagnosis of cerebro-spinal meningitis and sent to Castalia for Dr. Ladd.

Note by Dr. Ladd: Man of powerful build; comatose and restless: thrashing about the bed at times. Marked opisthotonos; purulent conjunctivitis; Kernig's sign present; petechial eruption and larger hæmorrhagic areas on body. Nose-bleed requiring packing to control. Temperature 103.5°, pulse 120, respirations 48 and stertorous. Lumbar puncture; 90 c.c. of turbid fluid removed; *10 c.c. of antiserum injected.* Regarded the condition as hopeless. April 5, in the morning patient still unconscious. Pulse was lower, temperature normal, respirations normal. In afternoon second puncture. 45 c.c. of less turbid fluid removed and *10 c.c. of antiserum injected.* April 6, *10 c.c. of antiserum injected.* Patient still unconscious. April 7, patient coming out of unconscious state. The progress was fluctuating and the patient was not without fever for 30 days. The improvement was gradual and the restoration complete. The patient returned to his work.

Discussion.—The case was one of abrupt and violent onset and of gradual subsidence of symptoms. The first puncture and injection of the serum were made on the fourth day of illness and were followed rapidly by improvement in the temperature, pulse and respirations. Two further injections of serum were given. The recovery was slow and complete. Total amount of serum injected, 30 cubic centimeters.

The epidemic of Castalia embraced 18 cases of meningitis of which 12 died and 6 recovered. Three of the latter recovered without lumbar puncture being performed and without the antiserum being injected. The three cases which were injected with the serum recovered. As these were the first cases in series to be injected with the serum the doses employed were smaller than the ones used subsequently.

The first case was injected with serum about 72 hours after the onset of the disease. Improvement was gradual and the symptoms subsided by lysis, hence it is not possible to assign certainly the

precise value of the serum injections. The second case, which was already in its second week of the disease, seems to offer more certain proof of the value of the puncture and serum injection. The disease terminated abruptly after the injection of 5 cubic centimeters of the serum and recovery was rapid and complete.

The third case was regarded as hopeless. Lumbar puncture and injection of antiserum having been made, the patient's condition changed quickly for the better, but the final recovery was fluctuating and gradual. The precise value of the serum injections must therefore remain doubtful.

EMPLOYMENT OF THE SERUM BY DR. L. W. LADD, OF CLEVELAND.

Dr. Ladd treated in all sixteen cases of epidemic meningitis in which the diplococcus was found with the antiserum in Castalia and Cleveland. He has kindly supplied us with the notes of his cases which will be presented in this place. Since three of the cases treated by Dr. Ladd were at Castalia the histories relating to them are given under the local epidemic of which they formed a part. We will first present Dr. Ladd's own analysis of the sixteen cases, then an analysis of our own, after which the case-histories of each to which we have added a brief discussion will be given.

"The sixteen cases consisted of 11 males and 5 females. Five patients were over 16 years, eight under 5 years and three between 5 and 8 years of age. When first seen thirteen of the sixteen cases were in coma, one was semi-comatose and two were conscious. Five patients were seen 24 hours or slightly earlier after the onset of symptoms. All these cases recovered completely. One patient was seen 48 hours after the onset. The condition was desperate. The patient died. Four patients were seen approximately 72 hours after the onset. They recovered. Two of these cases recovered completely, one developed foot-drop and recovered subsequently, one had impaired hearing when last seen. Three patients were seen approximately 96 hours after the onset. They died. Two of these cases showed a temporary improvement following lumbar puncture and serum injections. Two patients were seen two weeks after the onset, one recovered, the other died of chronic hydro-

cephalus. One patient was seen one month after onset and died of chronic hydrocephalus."

The percentage of recoveries among the sixteen cases was 68.75 and the deaths 31.75. Taking the ten cases treated with serum within 72 hours of the onset of symptoms, nine recovered and one died. The percentage of recoveries in this series is 90. Two of the five cases ending fatally were injected with the serum 96 hours or thereabouts after the onset and two cases several weeks after the beginning of the disease.

Case	I,	1st	injection	on	3d	day;	total serum injection	25 c.c.; recovery by lysis.
"	II,	"	"		12th	"	"	5 c.c.; " crisis.
"	III,	"	"		4th	"	"	30 c.c.; " lysis.
"	IV,	"	"		5th	"	"	15 c.c.; died.
"	V,	"	"		2d	"	"	35 c.c.; recovery by crisis.
"	VI,	"	"		1st	"	"	15 c.c.; " "
"	VII,	"	"		3d	"	"	20 c.c.; " "
"	VIII,	"	"		3d	"	"	45 c.c.; " lysis.
"	IX,	"	"		2d	"	"	35 c.c.; died.
"	X,	"	"		1st	"	"	15 c.c.; recovery by crisis.
"	XI,	"	"		20th	"	"	25 c.c.; died.
"	XII,	"	"		4th	"	"	46 c.c.; "
"	XIII,	"	"		1st	"	"	45 c.c.; recovery by crisis.
"	XIV,	"	"		14th	"	"	28 c.c.; " lysis.
"	XV,	"	"		3d	"	"	53 c.c.; " "
"	XVI,	"	"		4th	"	"	41 c.c.; died.

Scrutinizing this table closely we find that of the 11 patients who recovered the disease terminated, after serum injection, by lysis in five and by crisis in six cases. It is somewhat noteworthy to find that a case of meningitis which has lasted twelve days without intermission of symptoms should terminate abruptly after a lumbar puncture and serum injection. But of the several important cases terminating abruptly by crises where the serum injections were made on the first to third day, the most significant is case XIII in which the injection was made about two hours after the first appearance of the meningeal symptoms with the result of immediately arresting the progress of the disease.

A second tabulation dealing with the cases in children under five years of age, owing to the high mortality of epidemic meningitis in infants will be given.

Case II, Age	3 years;	recovered;	1st injection on	4th	day of illness.		
" V, "	3 "	"	"	2d	"	"	
" VI, "	2 "	"	"	3d	"	"	
" X, "	2 "	"	"	1st	"	"	
" XI, "	1½ "	died	"	20th	"	"	
" XII, "	2 "	"	"	4th	"	"	
" XIV, "	1½ "	recovered	"	14th	"	"	
" XVI, " under	5 "	died	"	4th	"	"	

Of the eight cases of this tabulation five recovered and three died. The three deaths occurred in children who received the serum for the first time on the twentieth and the fourth day of the illness respectively. On the other hand two children who were first injected on the fourth day, and one child injected on the fourteenth day recovered. There is no proof that the course of the disease in the last case was essentially influenced by the antiserum.

Case I. B. K. Castalia epidemic. Recovered.

Case II. F. W. Castalia epidemic. Recovered.

Case III. J. B. Castalia epidemic. Recovered.

Case IV. H. White male, aged 17 years. Barberton, Ohio.

The patient was employed at the Goodrich Rubber Co.'s factory at Akron. May 2, in the evening, he complained of fever and headache. He was seen by Dr. Lahmers May 3. At that time there were diarrhœa and vomiting, but no fever. The same evening meningeal symptoms and coma came on. May 6 Dr. Ladd saw the patient. There were extreme opisthotonos—the head almost touched the scapulæ—general rigidity, Kernig's sign, petechiæ over body, and purulent conjunctivitis. Temperature 103.5°, pulse 85, respirations 30. Lumbar puncture yielded two or three drops of thick pus. *15 c.c. of antiserum were injected.* No beneficial effect was noted. Died May 7.

Discussion.—The character of the exudate in this case and the general condition of the patient five days after the beginning of the infection, probably operated against any beneficial effects resulting from the serum. Total amount of serum injected, 15 cubic centimeters.

Case V. W. F. Male, aged 3 years. Bohemian.

On March 31 complained of headache in afternoon. April 1, severe headache; vomited. From 1 until 5 p. m. five convulsions, each lasting five minutes.—5 p. m. Coma, opisthotonos and Kernig's sign present; abdominal reflex absent; small petechiæ over body.—9 p. m. Lumbar puncture: 30 c.c. turbid spinal fluid removed and *10 c.c. of antiserum injected.* Temperature 99.4°, pulse 125, respirations 30.

April 2. Patient improved. Temperature and respiration unchanged, opisthotonos less marked, abdominal reflex present. Child conscious and rational; took nourishment.

April 6. Until to-day the child has done well. Temperature suddenly rose to 103.5°, pulse 116.

April 7. Lumbar puncture done in morning and 30 c.c. turbid fluid removed and *15 c.c. of serum injected*. The child was delirious before the puncture.

April 8. Temperature 99.6°, pulse 120.

April 22. Child did well until to-day, when the temperature rose to 103.2°; pulse 132. Vomiting and meningeal symptoms present. Lumbar puncture removed 30 c.c. of fluid; *10 c.c. of antiserum injected.*

April 24. Temperature normal. From this date on the recovery was uneventful and finally was complete.

Discussion.—The first lumbar puncture and serum injection were made within 48 hours of the onset of symptoms. The disease seemed to have been promptly arrested by the injection. Subsequently two relapses occurred, one on the sixth and the other on the twenty-second day, which were as abruptly arrested as the first symptoms by the lumbar puncture and serum injection. Recovery was complete. Total amounts of serum injected, 35 cubic centimeters.

CASE VI. Baby N. Female, aged 2 years. Italian.

Child lived in the poor district of Cleveland. Dr. Steuer, the attending physician, diagnosed the case as meningitis and called Dr. Ladd—22 hours after the onset of symptoms. Child had marked opisthotonos, lateral nystagmus, Kernig's and MacEwen's signs and muscular rigidity. She was completely comatose. Temperature 102.5°, pulse 140, respirations 40. Lumbar puncture: 50 c.c. of turbid fluid removed and *15 c.c. of antiserum injected.* Morning of next day, temperature, pulse and respirations normal; child dull and listless but conscious. Kernig's sign and opisthotonos less marked. Parents refused second puncture and injection of serum. The meningeal symptoms quickly subsided and recovery was complete. A few days later whooping cough developed, from which recovery was finally made.

Discussion.—The essential facts in this case are the sudden onset of severe symptoms of meningitis in an infant of two years and their abrupt arrest and permanent and rapid dissipation after lumbar puncture and serum injection performed in the first first twenty-four hours of the disease. Total amount of serum injected, 15 cubic centimeters.

CASE VII. X. Female, aged 11 years. American.

May 1. Patient seized with severe headache, vomiting, stiffness of neck. 12 hours later unconscious. Opisthotonos marked; Kernig's sign present; lateral nystagmus; petechial eruption. May 4 Dr. Ladd saw patient and did lumbar

puncture. 30 c.c. of turbid fluid removed and *10 c.c. of antiserum injected* (this within 72 hours of the onset of the symptoms). Temperature 103.6°.

May 2. Temperature and pulse normal; patient answered questions rationally though lapsed into semi-consciousness when left alone.

May 6. Improved.

May 7. Temperature rose and meningeal symptoms became prominent. Lumbar puncture repeated; *10 c.c. antiserum injected.* Temperature fell to normal. Complete recovery.

Discussion.—The prompt amelioration of the severe symptoms by the first lumbar puncture and serum injection and the equally prompt suppression by these means of what threatened to be a relapse of the disease, are striking incidents of this case. Total amount of serum injected, 20 cubic centimeters.

Case VIII. W. H. White male, aged 21 years. Hudson, Ohio. Farmer.

May 12. Had a chill in evening. May 13. Unable to work.—8 a. m. Had a second chill and severe headache. In a few minutes was delirious and soon became unconscious. Dr. Ladd saw the patient May 14. There were present marked opisthotonos, Kernig's sign, general muscular rigidity and petechial eruption. (It developed that two weeks before the patient's mother had nursed a fatal case of epidemic meningitis.) Lumbar puncture was attempted four times but only a few drops of bloody serum were obtained. This fluid showed doubtful diplococci. The culture was negative. Clinically there was no doubt of the diagnosis. In view of the fact that no spinal fluid was obtained, *5 c.c. of antiserum* were injected into the canal and *10 c.c. antiserum under the skin.* Temperature 103.5°.

May 15. Patient improved; rational at times, opisthotonos, muscular rigidity and Kernig's sign less marked. *15 c.c. antiserum injected subcutaneously.* The temperature ranged from 100° to 103°, when, on the twelfth day, it fell to normal and remained there. On the seventh day of illness another dose of *15 c.c. of the antiserum* was given subcutaneously. A marked erythematous rash appeared on the fifteenth day. Recovery was complete.

Discussion.—The absence of bacteriological confirmation of the diagnosis in this case is an evident weakness, but there would appear to be little doubt of the nature of the disease. What is noteworthy is the subcutaneous employment of the antiserum. The symptoms gradually subsided by lysis and hence the special influence of the serum cannot be defined with certainty. Total amount of serum injected, 45 cubic centimeters.

Case IX. C. G. White female, aged 6 years.

The patient was one of three children in one family afflicted with meningitis. June 30, at 4 a. m., was taken with headache and vomiting. July 1, at 10 a. m.,

had a convulsion. She was brought comatose to the City Hospital. There were marked Kernig's sign and opisthotonos. Extensive petechiæ and herpes oris. July 2, 8 p. m., lumbar puncture yielded 5 c.c. very turbid fluid. *10 c.c. of antiserum were injected.*

July 3, 10.30 a. m. Lumbar puncture done and 15 c.c. very turbid fluid removed. *10 c.c. antiserum injected intraspinally and 5 c.c. subcutaneously.*

July 4. Right-sided hemiplegia developed. Patient's condition still very bad.

July 5, 11 a. m. Lumbar puncture and administration of *10 c.c. antiserum.*—7 p. m. Condition very bad. Temperature 107.4°. Death. Consciousness never regained.

Discussion.—The onset of symptoms was in this case rapid and severe. The first lumbar puncture and serum injection were made about 64 hours after the first symptoms were noted, and while the patient was in an unconscious state. Two later injections of the serum were given, but no beneficial effect followed any of the injections. Total amount of serum injected, 35 cubic centimeters.

CASE X. J. C. White male, aged 2 years.

This is the second child having meningitis in this family. July 1, patient put to bed in good health. Later in the evening he was found restless and feverish. The physician made a diagnosis of meningitis. Admitted to City Hospital July 2, 9 a. m.

July 2. On admission marked opisthotonos, Kernig's sign and general petechial eruption were noted. Child comatose. At 4 p. m., approximately 20 hours after onset of symptoms, lumbar puncture performed and 50 c.c. of turbid fluid removed; *10 c.c. of antiserum injected into spinal canal and 5 c.c. under the skin.* Temperature at this time was 104°, pulse 140, respirations 62.—7 p. m. Temperature 105.6°, pulse 148, respirations 78.

July 3. This morning temperature 100°, pulse 128, respirations 40. Child conscious and irritable.

July 4, 10.30 a. m. Lumbar puncture negative (probably unsuccessful). The patient's condition quickly improved and, except for a slight rise of temperature to 102.4° at 12 a. m. July 5, the temperature, pulse and respirations remained normal.

July 17. No complications have developed. Child discharged cured.

Discussion.—The onset of symptoms in this case was sudden and severe. Twenty hours after the symptoms appeared lumbar puncture and serum injection were performed. Within twenty-four hours of these operations the condition had changed abruptly for the better and the disease had terminated by crisis. Recovery was uninterrupted. Total amount of serum injected, 15 cubic centimeters.

CASE XI. P. G. White male, aged 18 months. Austrian.

March 12. Admitted to Lakeside Hospital. Until March 4 the child had been well since birth. On that day was fretful and feverish, coughed and had diarrhœa. March 7, right elbow swollen and painful; neck stiff; macular eruption over body. On admission to Hospital there were irritability, large head retracted to right side, slight strabismus, pupils equal and active to light, slight swelling about flexed and rigid right elbow.

March 23. Temperature 105°. Lumbar puncture: 25 c.c. slightly turbid spinal fluid removed. On microscopical examination a small number of leucocytes containing diplococci of typical intracellularis appearance seen. No change in condition as result of puncture. Lumbar puncture every third or fourth day as routine. Temperature ranged from 98° (a. m.) to 104° (p. m.). No marked change in the physical condition.

April 12. Lumbar puncture and *intraspinal injection of 5 c.c. serum.*

April 14. Temperature did not rise above 100.5°, which was the lowest maximum temperature since admittance.

April 16, a. m. Temperature normal. *7 c.c. antiserum injected.* Temperature normal until April 23, when it rose suddenly to 104°, pulse 140, respiration 40. Symptoms of chronic hydrocephalus developed. Several additional punctures and injections of serum were made without beneficial effect. Death on July 30.

Discussion.—The first lumbar puncture was made on the twentieth day and the first serum injection was given on approximately the fortieth day of illness. Following the latter the symptoms diminished and there seemed decided improvement lasting eleven days. The symptoms reappeared and death resulted from chronic hydrocephalus. Total amount of serum injected, more than 25 cubic centimeters.

CASE XII. F. P. White male, aged 3 years.

Onset sudden on May 18 with headache, vomiting and temperature to 106.5°. Bright red pin-point rash over body. May 20, first convulsion noticed. Opisthotonos pronounced.

May 22 (about 96 hours after onset). Lumbar puncture and 45 c.c. of very turbid fluid withdrawn. Two hours later second puncture, 20 c.c. fluid removed and *15 c.c. antiserum injected.* Temperature 103°.

May 23. Patient brought to Lakeside Hospital. Temperature 101°, pulse 140, respirations 40. Conscious, very irritable, opisthotonos and Kernig's sign moderately marked (as on 22d, when seen by Dr. Ladd). Petechial eruption present.—4 p. m. Lumbar puncture: 50 c.c. turbid fluid withdrawn and *15 c.c. antiserum injected.*—12 a. m. Temperature 101.6°.

May 24. Patient drowsy; neck rigidity increased. 8 a. m. Temperature 102°. —3 p. m. Lumbar puncture: 40 c.c. turbid fluid removed and *16 c.c. serum injected.* Temperature 103.5°, pulse 135.—4 p. m. Chill, marked cyanosis, pulse feeble, respirations poor.—4 p. m. Temperature 106°. After tub bath temperature 104°.—9 p. m. Chill, cyanosis, temperature 106.5°. Death at 1.25 a. m., May 25.

Discussion.—The first lumbar puncture and serum injection were made 96 hours after the onset of symptoms, and on the next two successive days the punctures and injections were repeated. The disease progressed rapidly and continuously and ended fatally approximately six days after the onset. The question arises whether the sudden change for worse on May 24 bore any relation to the puncture and injection of serum one hour previously. There are no data at hand to use in answering this question. In other cases in which daily injections of serum were made severe symptoms did not appear. Total amount of serum injected, 46 cubic centimeters.

CASE XIII. O. C. White female, aged 7 years. Sister of F. C., Case XII.

Until May 21 child well. Symptoms began with fever (to 106°), severe headache, thirst and erythematous eruption over arms and legs. May 22, 2 p. m., child seen by Dr. Ladd.

May 22. Child conscious. No Kernig's sign. No opisthotonos, rigidity of neck or extremities. A bright erythematous eruption over chest and extremities. During the two hours which were employed in the examination of the fluid withdrawn from brother, marked meningeal symptoms developed, convulsions and opisthotonos and coma set in. Lumbar puncture was done, 30 c.c. of turbid fluid were withdrawn, and *15 c.c. of antiserum injected intraspinally.* The patient spent a quiet night and was brought to the Lakeside Hospital May 23.

May 23, a. m. Temperature 100°, pulse 120, respirations 35. Considerable restlessness. Moderate opisthotonos and rigidity of extremities. Unconscious. Kernig's sign present.—4 p. m. Lumbar puncture: 25 c.c. serum under moderate pressure removed and *15 c.c. serum injected.*

May 24. Condition greatly improved. Opisthotonos almost gone. Kernig's sign diminished, child less irritable and conscious though dull. Temperature normal, except for rise to 101.5° following puncture and injection of *15 c.c. of antiserum.* Only a few drops of turbid fluid obtained.

May 25. Temperature normal; mind clear; meningeal symptoms absent. Recovery uninterrupted. The disease in this case terminated in four days from its beginning.

Discussion.—The symptoms in this case set in abruptly and with much intensity. Within the first 24 hours of illness the diagnosis was established by lumbar puncture and the first serum injection made. What is especially important in this case is the fact that the puncture and serum injection were made in less than two hours after the appearance of symptoms due to meningeal irritation. The disease appears to have been arrested by the first puncture and

injection and, although two subsequent injections of serum were made, the symptoms disappeared rapidly after the first injection and the patient was well on the fourth day from the onset of the illness. Total amount of serum injected, 45 cubic centimeters.

CASE XIV. L. S. White male, aged 1 year and 7 months. Pole.

May 25. Admitted to Lakeside Hospital. Two weeks before the child fell while playing and lay for a few moments with head retracted. In a little while fell again in convulsion. On undressing him the mother noticed an eruption on the body. During the next five days the child had four convulsions; has been in bed since with head retracted and squint. On admission to hospital there were noted emaciation, marked opisthotonos and internal squint. Kernig's sign present. Lumbar puncture gave 25 c.c. turbid fluid. Temperature 101°, pulse 165, respirations 45. *5 c.c. antiserum injected.* Temperature normal during next four days.

May 28. Temperature 101.5°. Lumbar puncture gave 15 c.c. clearer fluid. *6 c.c. antiserum injected.* From the first puncture and injection the opisthotonos diminished and the mental condition brightened. Until June 1 temperature normal.

June 1, 12 a. m. Temperature 103.5°; next morning normal. Child gaining steadily in weight.

June 12. Temperature 103°. Lumbar puncture yielded 25 c.c. of clear spinal fluid in which a small number of diplococci were still present. *7 c.c. antiserum injected.* Until June 25 steady improvement.

June 25. Temperature 102°. *5 c.c. of serum given under the skin.* Temperature returned to normal but on June 27 again rose to 102°. *5 c.c. serum given subcutaneously.* No further rise occurred. Discharged cured.

Discussion.—This case is an example of sub-acute epidemic meningitis in which the symptoms rapidly ameliorated after lumbar punctures and serum injections began two weeks after onset of the disease. The only point that is especially noteworthy is the rapid clearing of the spinal fluid after the first puncture and serum injection. No statement of the precise part played by the serum can be made. Total amount of serum injected, 28 cubic centimeters.

CASE XV. C. D. White male, aged 8 years. German.

The patient was well until the night of June 13. Onset of disease was with irritability, severe headache and vomiting. June 16, diagnosis of meningitis made. There were present marked opisthotonos, muscular rigidity of extremities and petechial eruption. Patient comatose, respirations stertorous.

June 16, 1 p. m. Temperature 103°. Lumbar puncture gave 45 c.c. of turbid fluid; *10 c.c. antiserum injected.*—8 p. m. 45 c.c. spinal fluid removed and *5 c.c. antiserum injected.* The symptoms ameliorated somewhat during the day.

June 17. Temperature and pulse normal. Toward evening patient can be made to respond to loud questions but answers are unintelligible.

June 18, a. m. Temperature 104°; restless.—9.50 p. m. Lumbar puncture: 40 c.c. of turbid fluid withdrawn and *15 c.c. of antiserum injected.*

June 19, 12 a. m. Patient quiet, temperature normal. Mental condition clearer, temperature not above 101.5° for two days.

June 21. Restlessness; temperature 104°. Lumbar puncture: no fluid obtained, *5 c.c. antiserum injected under the skin.*

June 22. Temperature normal.

June 25. *5 c.c. serum subcutaneously.*

June 26, 12 a. m. Temperature 103°.

June 27, 8 a. m. *5 c.c. serum subcutaneously.* Temperature remained below 102° until June 30, when it rose suddenly to 104°.

June 30 to July 8. Up and down temperature.

July 8. Owing to increased irritability and symptoms of meningeal irritation lumbar puncture made and 35 c.c. of nearly clear fluid withdrawn.

July 10. After two days of nearly normal temperature there was a rise to 105.8°. Lumbar puncture made and *8 c.c. antiserum injected.*

July 11. Temperature normal. No further rise from this time. Patient discharged on July 29 well except for slight impairment of hearing.

Discussion.—The first puncture and serum injection were made on the third day of the disease and were followed by improvement in the patient's condition. The course of the illness was fluctuating and the symptoms subsided gradually and with occasional exacerbations. The lumbar punctures and serum injections, made during the access of the symptoms, appeared to control the fever and reduce the restlessness, while the subcutaneous injections of the serum would appear to have been less effective in this respect. Recovery was incomplete as impairment in hearing remained. Total amount of serum injected, 53 cubic centimeters.

Case XVI. I. L. White, aged under 5 years. Hebrew.

June 18. Until to-day well. Complains of headache. June 19. Severe headache, vomiting, opisthotonos, petechial spots on body. In the evening unconscious. June 22. Admitted to Hospital (approximately 96 hours after onset). Symptoms unchanged except for exophthalmos on the right side and a lateral nystagmus. Lumbar puncture yielded 30 c.c. of very turbid fluid. *6 c.c. of antiserum injected.* Temperature fell from 104° to 100° in four hours; muscles less rigid; mind clearer.

June 23, a. m. Spinal puncture: 30 c.c. fluid removed and *5 c.c. serum injected.*

June 24, 4 p. m. Spinal puncture: 35 c.c. fluid removed and *5 c.c. serum injected.*

June 25. *5 c.c. serum given subcutaneously.* Following this the temperature which had ranged from 104.5° to 100° fell to normal.

June 27, 8 a. m. Temperature rose to 103°. Lumbar puncture, 2.5 c.c. reddish serum obtained and *5 c.c. serum injected.* Until July 9 child seemed to improve. The opisthotonos had diminished; temperature slightly elevated, sometimes reaching 104°.

July 9. Lumbar puncture: 45 c.c. of fluid removed and *5 c.c. of antiserum injected.*

July 13. 37 c.c. fluid withdrawn by puncture and *10 c.c. serum injected.* The temperature remained fluctuant; symptoms of increasing hydrocephalus developed and punctures and serum injections were repeated, but death took place on July 31, 1907.

Discussion.—This child was first injected with serum on the fourth day of the illness after which the acute symptoms ameliorated somewhat. The progress of the disease was not arrested by the several punctures and serum injections and chronic hydrocephalus and death resulted. Total amount of serum injected exceeded 41 cubic centimeters.

THE CASES OF EPIDEMIC MENINGITIS AT PHILADELPHIA.

The cases of meningitis at Philadelphia followed on the heels of the large epidemic at New York and probably constituted part of that epidemic. The report of the use of the antiserum at the Pennsylvania Hospital, supplied by Dr. Longcope, embraces five cases of the disease. Of these, four recovered and one died. We will tabulate these cases according to the period of the first injection of the serum, the total amount of antiserum employed in each case, and the mode of termination of the disease.

Case	I,	1st	injection	on	4th	day;	total serum injection	15 c.c.;	recovery by crisis.
"	II,	"	"	3d	"	"	"	25 c.c.;	" lysis.
"	III,	"	"	4th	"	"	"	45 c.c.;	" crisis.
"	IV,	"	"	11th	"	"	"	25 c.c.;	death.
"	V,	"	"	10th	"	"	"	15 c.c.;	recovery by lysis.

Pennsylvania Hospital, Philadelphia. Service of Dr. M. J. Lewis.

CASE I. J. J. Negro male, aged 23 years. Stevedore.

Present Illness.—On the morning of July 2, 1907, the patient experienced headache, indigestion, weakness and exhaustion and pain in the back. During the afternoon the headache increased in severity, and a sense of malaise and illness was felt. He was brought to the receiving ward by the patrol service and remained over night. The next morning, as he felt better, he left the hospital and went to the wharf and lay down. Soon afterwards he became nauseated and vomited freely. The pain in the head returned with increased severity and there were pain and stiffness of the back of the neck, chilly and feverish sensations and

general pain of the body. That night (July 3) the patient went home and to bed and in spite of being very ill he went to work the next day. Later he was found unconscious on the wharf and was brought to the hospital (July 4). The note on admission states that the patient is unconscious, the neck is rigid, the urine is voided involuntarily, the temperature is 101° F., the pulse 80, and the respirations 26.

Physical Examination.—Large, robust negro lying unconscious but restless; irrational and somewhat noisy. Skin hot and dry; no rash; pupils react equally to light; slight degree of nystagmus and strabismus; conjunctivæ injected; tongue moist and heavily coated; breath offensive; moderate degree of rigidity; Kernig's sign present; patellar reflexes practically absent; sensations appear to be normal. (Nothing abnormal was discovered in heart, lungs and abdomen.) The urine showed a faint trace of albumen and a small number of hyaline and granular casts and leucocytes, but no sugar.

July 5. The alterations since yesterday are increased rigidity of neck and more marked Kernig's sign. Leucocyte count 15,800.—1 p. m. Lumbar puncture: 20 c.c. of very turbid fluid showing many *Diplococcus intracellularis* and pus cells obtained.—5 p. m. Second puncture: 65 c.c. fluid evacuated and *15 c.c. antimeningitis serum injected.* Temperature 100° F.—6 p. m. Temperature 102.2°, respirations increased, some degree of relaxation, patient quieter and less delirious apparently.—6.30 p. m. Temperature 101°, pulse 96, respirations 32.—9 p. m. Temperature 99.3°, pulse and respiration improved, patient more relaxed and has been perspiring, pupils react to light.—10.40 p. m. Partly rational and can be aroused sufficiently to give name, address and occupation; relaxation still more marked; headache is complained of.

July 6. Condition improved. Temperature 98.3°, pulse 60, respiration 20. Neck slightly rigid only, Kernig's sign greatly diminished; pupils equal and react; conjunctivæ clear; nystagmus and strabismus diminished; patient quiet and rational but is drowsy and somewhat stuporous. The next note reads: "This evening the temperature has gone up again; otherwise the general condition is much improved."

July 7. Temperature normal; condition improved; symptoms abating; headache, strabismus and muscular rigidity less marked; some diplopia present. Bowels moved freely.

July 8. Temperature 100°, otherwise no important change.

July 9. The note reads: "Temperature, pulse and respiration are normal. The patient is much brighter, headache has almost disappeared, the body is almost perfectly relaxed, patellar reflexes are normal, there is only a faint suggestion of Kernig's sign, the diplopia is less marked, but there is strabismus and paralysis of the left external rectus. The bowels have moved and the patient complains of hunger."

July 10. The urine contains a trace of albumen but no casts. Leucocytes 7,160.

July 11. Condition good, no discoverable rigidity, no headache, patient bright and hungry, tongue clean, temperature normal. Allowed to sit up.

July 12. All the symptoms except paralysis of external rectus and slight degree of diplopia have subsided; a suggestion of Kernig's sign remains. The diet is increased and the patient is allowed out of bed for a few minutes.

July 15. About ward.

July 18. Diplopia and Kernig's sign seem to be disappearing; still some paralysis of external rectus.

July 21. Feels well; can read without difficulty. No headache.

July 24. Improvement continues, paralysis of external rectus diminishing; all other symptoms have subsided.

July 26. "Discharged cured."

Discussion.—The first symptoms of meningitis appeared on July 2, and it is probable that lumbar punctures made at that time might have developed the nature of the disease. The symptoms increased in severity progressively until July 4 when they became marked. In view of the almost unavoidable uncertainty attaching to a determination of the onset of the disease it is safe to count July 3 to 4 as the first twenty-four hour period of its actual existence. Hence the serum was injected about 48 hours after the development of unmistakable symptoms. The symptoms abated rapidly after the second lumbar puncture and the injection of serum, and the patient experienced no relapses but improved progressively and made a complete recovery. Total amount of serum injected, 15 cubic centimeters.

Pennsylvania Hospital, Philadelphia. Service of Dr. J. C. Wilson.

CASE II. R. B. White male, aged 17 years. Clerk.

Present Illness.—On June 27, 1907, the patient was brought to the hospital in ambulance. He complained of malaise, severe headache, vomiting and constipation. The illness began on June 25 with malaise and headache. The same night the symptoms became suddenly much worse: headache, severe aches and pains of body, nausea and vomiting, chilly and feverish sensations, and alternating delirious and lucid periods until morning. The symptoms continued the next day and rigidity of neck was complained of. On admission temperature 100°, pulse 100, respirations 28.

Physical Examination.—Well-developed, robust-looking boy. Lies quietly in bed, although he tends to be restless and irritable; is rational but stuporous. Skin dry and hot; no rash; no herpes; face flushed; pupils slightly dilated and equal; react; conjunctivæ clear; tongue heavily coated and dry; head slightly retracted and rigid and painful. Kernig's sign marked; suggestion of Babinski reflex; patellar reflexes practically absent; some cutaneous hyperæsthesia; leucocytes 16,500 (nothing abnormal was discovered in heart, lungs and abdomen).

June 28. Condition essentially unchanged. The head is held more rigidly possibly and much headache is complained of.

June 29. Herpes appeared on lips; lumbar puncture at 3 p. m. and moderately turbid fluid obtained. *10 c.c. of antimeningitis serum injected* slowly into canal. No microorganisms found in the spinal fluid. One hour after the injection the temperature dropped from 100.6° to normal, the pulse became softer and ranged

from 74 to 64; respirations unchanged. Rigidity unchanged. In the evening temperature rose to 99.6°; patient very restless; morphia administered hypodermically.

June 30. Temperature 100.4°, dropping later to 98.4°; patient somewhat more comfortable; pulse 72. Evening: patient seems better, less pain and rigidity of head; has been sleeping or dozing much of the time; takes nourishment; bowels freely opened. Leucocytes 15,800.

July 1, 4.30 p. m. Lumbar puncture and 80 c.c. fluid removed; *15 c.c. antimeningitis serum injected.*—7 p. m. Temperature has risen from 98.5° to 101.5°. Patient perspired freely. No microorganisms found on cover-slips but many leucocytes. One colony of Gram-negative diplococcus grew on calf-serum agar.

July 2. Kernig's sign present; pain in back and legs; no headache; rigidity of head diminished. Leucocytes 10,550.

July 3. Has been very comfortable; no morphia given since July 1; no headache; rigidity of neck much lessened so that the head can be brought pretty well forward. Kernig's sign much diminished; no bodily discomfort; temperature normal or slightly subnormal.

July 4. Quite comfortable; hungry; given ice cream and orange juice. Kernig's sign diminishing.

July 6. Slight evidence of Kernig's sign; comfortable; on soft diet.

July 9. Up on bed rest.

July 16. Stiffness and pain in neck entirely and Kernig's sign practically gone. Temperature subnormal. Discharged.

Discussion.—The disease in this case began, probably, on June 25, the symptoms growing severe within 24 hours of their first appearance. The diagnosis of epidemic meningitis is rendered probable but not certain by the bacteriological examination. Lumbar puncture was performed and anti-meningitis serum injected twice. The first injection was made on approximately the third day, and the second on the sixth day of the disease. The recovery of the patient was progressive and complete without relapses.

Pennsylvania Hospital, Philadelphia. Service of Dr. Newlin.

Case III. A. G. White female, aged 15 years. Canvas weaver.

Present History.—Went to work on September 6, but came home at noon on account of pain in head and back of neck. September 7 and 8 vomited several times. Since 6th inst. has been delirious and feverish and crying out. Admitted to hospital September 8.

Physical Examination.—Well-developed young girl lying in bed with chin thrown up and neck held stiffly; eyes injected; pupils equal, react; tongue heavily coated, dry; herpes about mouth; crying out; no rash. Kernig's sign well marked; knee jerks present, no ankle clonus. Nothing abnormal discovered in heart, lungs and abdomen.

September 9. Lumbar puncture yielded 3 c.c. of very turbid fluid containing *diplococcus intracellularis.* Leucocytes 23,000.

September 10. Condition unimproved. Kernig's sign and rigidity of neck more marked. Patient delirious, but can be made to give rational answers to questions.

September 11. Leucocytes 12,200. 50 c.c. of turbid, white fluid containing meningitis cocci withdrawn by lumbar puncture and *15 c.c. antimeningitis serum injected* into the spinal canal.

September 12. The note reads: "Twelve hours after the puncture and injection of the serum the temperature came down by crisis from 102.5° to 97° F.; the patient was quiet and sleeping soundly. The pulse was good and the respirations slow and regular. This morning the rigidity of the neck is lessened, the headache is less, the temperature subnormal, the respirations are regular and slow, the pulse slower (84) and of good volume, and the patient is improved in every way. The pupils are moderately dilated and the movements of the eyes good. There is a marked herpetic eruption about the mouth and three ulcers; one between fingers on the left hand and one on the left ear, and another beginning beneath the left eye. Leucocytes 8,750. "While counting the leucocytes six diplococci were found in a broken polymorphonuclear cell" (note by Dr. Longcope).

September 13. The temperature rose again yesterday, but came down during the night. This morning obtained 30 c.c. fluid by lumbar puncture and injected *15 c.c. antimeningitis serum.*

September 14. Much better to-day. Rigidity of neck and Kernig's sign almost gone; temperature subnormal; is rational.

September 15. Improving, but paralysis of the left external rectus is noted.

September 16. The paralysis of external rectus more noticeable and slight rigidity of neck and Kernig's sign still persist. 35 c.c. of clear, colorless fluid containing a few whitish flakes but showing no diplococci were obtained on lumbar puncture. *15 c.c. of antimeningitis serum injected.* Leucocytes 8,800. In the evening the temperature rose to 101°, the rate of respiration increased somewhat, but the pulse continued good (108). The rigidity of the neck and Kernig's sign were both increased, but they were much less marked than on admission.

September 18. Condition much improved. Neck rigidity and Kernig's sign have disappeared. Patient is hungry.

September 20. Temperature normal for three days.

September 24. Improving daily; house diet.

September 26. Out of bed.

September 30, discharged cured.

Discussion.—The onset of the disease in this case was abrupt, and the patient was admitted to the hospital about 48 hours after the appearance of symptoms. The diagnosis was established by the clinical symptoms and by lumbar puncture and a bacteriological examination on the third day of the disease, and a second puncture and an injection of the serum were made on the fourth day of the disease. It is worth noting that the circulating leucocytes fell to

one half the number between the first two punctures. A critical fall in the temperature took place after the second puncture and the first injection of the serum, and the general condition of the patient changed for the better while the rigidity of the neck muscles diminished. As the temperature rose slightly 72 hours after the first injection of serum a second puncture and serum injection were made. No further rise in temperature is recorded following the second injection of the serum. But as Kernig's sign and stiffness of the neck still persisted on September 16 and paralysis of the left external rectus appeared on the 15th, a third puncture and injection of serum were carried out. Since the leucocyte count was 8,800 on the day of the last injection complete previous cessation of active inflammation may be assumed, and the assumption is rendered certain by the limpid character of the spinal fluid withdrawn, from which all diplococci had disappeared. Forty-eight hours after the third puncture and serum injection all symptoms had subsided. The patient made a rapid and complete recovery.

Pennsylvania Hospital, Philadelphia. Service of Dr. Alfred Stengel.

CASE IV. C. B. White male, aged 11 years. Italian.

Present Illness.—The patient was admitted to the hospital on October 1 and had been sick since September 21. The onset was accompanied by vomiting, fever and chills. The complaint is of headache and pain in the back of the neck; the patient is delirious.

Physical Examination.—Half-grown, fairly well-nourished boy. He is very restless, requiring to be strapped in bed, and he cries out in delirium. Eyeballs are prominent; herpetic eruption about nose and mouth; tongue rough and covered with a brownish coat. The head is retracted and efforts to move it forward cause him to cry out. Suggestive Kernig sign; knee jerks unsatisfactory. Temperature on admission 101.2° F.

October 1. Leucocytes 10,500. 3 p. m. Lumbar puncture unsuccessful.—12.30 a. m. Second futile attempt to secure fluid by lumbar puncture. Patient very restless. Chloral and sodium bromide administered but ineffectual. Fairly quiet for 5 or 6 hours after ethyl chloride.

October 2, 4 p. m. Lumbar puncture yielded 6 c.c. thick, viscid, yellow pus. The last amount withdrawn thinner than the first. *10 c.c. of antimeningitis serum injected.* Pus contains many meningitis cocci. Kernig's sign positive. Patient very restless; muscular twitchings.

October 3. Leucocytes twenty hours after puncture 14,750. No relief from puncture and serum. Three hours after puncture temperature rose from 101° to 104° F. It remained at 104°, except for a temporary drop to 102° through the night and the next morning. Marked nystagmus this morning. Pulse weak and rapid (130–160), respiratory rate has been steadily increasing; muscular

twitchings are very marked and the arms are jerked spasmodically. Stimulation increased, but pulse and respiration grow steadily more rapid and feeble.—4 p. m. Lumbar puncture yielded 12 c.c. turbid, purulent fluid. *15 c.c. of antimeningitis serum injected.* Pulse and respiration gradually increasing. Temperature at 8.15, 106°. Unconscious and less noisy in delirium. Died at 1.45 a. m. (October 4). The autopsy (Dr. W. T. Longcope) showed acute cerebro-spinal leptomeningitis, acute broncho-pneumonia; chronic interstitial hepatitis; chronic interstitial splenitis; chronic fibrous pleurisy; congestion of intestine and hyperplasia of lymphoid follicles; and cloudy swelling of the kidneys.

Discussion.—The patient was admitted to the hospital on the tenth day of the disease. The puncture of the spinal canal indicated, and the autopsy proved, the cerebro-spinal meninges to be covered with a thick layer of pus cells and fibrin. No favorable influence was exercised on the course of the disease by the puncture and the serum injection carried out first on the eleventh and next on the twelfth day of the disease when the patient was already in an extreme condition.

Pennsylvania Hospital, Philadelphia. Service of Dr. Henry.

CASE V. P. C. Male, aged 18 years. Italian. Shoemaker.

Present Illness.—Patient came to the hospital on August 7 complaining of malaise, severe headache, chilliness and fever, nausea and constipation. The headache and malaise began on August 4 and increased so that he was compelled to stay in bed. On admission to the hospital the temperature was 101°, pulse 72, respirations 24.

Physical Examination.—Patient is moderately well-developed. He lies quietly in bed but is stuporous. Skin dry and hot; no rashes. Somewhat flushed and apathetic facies. Pupils equal and react sluggishly. Conjunctivæ injected. Tongue heavily coated and moist; breath fetid. The patient resents disturbance and becomes irritable; complains of pain in the head. (Nothing abnormal discovered in heart, lungs and abdomen. A blood examination showed Widal test negative and leucocytes 18,700. Slight degree of Kernig's sign present. Urine: no albumen, sugar or casts.

August 9. Temperature has almost reached normal; headache severe; there is marked rigidity of neck with slight retraction of the head. Some degree of rigidity of the back. The reflexes are exaggerated. Lumbar puncture was made at 1 p. m. but no fluid was obtained.

August 10. Temperature has risen. The condition of patient apparently worse; the stupor has increased and at times there is delirium. Rigidity of neck and Kernig's sign have increased; slight degree of nystagmus and strabismus. Lumbar puncture at 1 p. m. yielded 3 c.c. of spinal fluid tinged with blood. The centrifugated specimen showed polymorphonuclear leucocytes and red corpuscles, but no bacteria. Cultures were negative.

August 12. Condition essentially unchanged. The temperature fluctuated somewhat. Complains less of headache. Leucocytes 19,750. Lumbar puncture

at 1 p. m. yielded 4 c.c. of fluid of which 3 c.c. collected in one tube was slightly blood-tinged and 1 c.c. in another presented a silvery sheen. Cover-glass preparations showed many polynuclear cells, a few mononuclear cells and many red corpuscles, but neither tubercle bacilli nor diplococci. Cultures were sterile.

August 15. Patient slightly improved; brighter; temperature lower; still has considerable headache; persistence of neck rigidity and Kernig's sign; patellar reflexes exaggerated. Pulse good.

August 16. Condition about the same except that the temperature has become normal: Lumbar puncture at 1 p. m. yielded 7 c.c. of turbid spinal fluid. No tubercle bacilli found, but many pus cells and extracellular and smaller number of intracellular diplococci resembling the intracellularis, were seen. At 2.45 p. m. *15 c.c. of antimeningitis serum injected into the spinal canal.* "Patient stood the ordeal well, one hour later felt more comfortable and was dozing."

August 17. Temperature remains normal. Patient feels much better; he is brighter; headache less severe; neck less rigid, relaxing; Kernig's sign diminished.

August 19. Patient Convalescing. Headache slight; rigidity less; appetite improved.

August 22. Condition good; no headache; no rigidity; reflexes normal. Sitting up.

August 30. Discharged cured.

Discussion.—The diagnosis in this case must be accepted chiefly, if not entirely, upon the basis of the symptoms. The bacteriological examination of the fluid yielded by lumbar punctures does not supply convincing proof of the existence of epidemic meningitis. The degree of general leucocytosis is in agreement with what is commonly found in epidemic meningitis, but the evidence supplied by the leucocyte count is circumstantial. The examination of the sediment obtained from the spinal fluid showed an excess of polymorphonuclear cells, but it was not until the 16th instant, or on the tenth to twelfth day of the disease, that turbid spinal fluid, containing many leucocytes and stainable diplococci, was secured. It does not appear in the report that the cocci were certainly identified as *Diplococcus intracellularis* by culture tests. Hence the question remains open whether in this case the general cerebro-spinal fluid remained free of the diplococcus although an exudative inflammation of some extent existed in the membranes. The case appears to have been progressing towards recovery before the injection of the serum on the tenth to twelfth day of illness, and there is lacking all certain evidence that the progress in this direction was accelerated appreciably by the serum. Total amount of serum injected, 15 cubic centimeters.

RESULT OF THE USE OF ANTISERUM BY DR. CUSHING, OF BALTIMORE.

Dr. Cushing wrote one of us (Flexner) as follows:

"I remember your telling me that you thought there was very little chance of benefit to be expected from the serum in other than the acute stages of epidemic meningitis. It may, therefore, interest you to know of this single experience. A woman, aged 36 years, had a severe attack of cerebro-spinal fever with sudden onset, May 16, 1907. She was very ill and during the next few weeks Futcher and a number of others saw her in consultation. The condition dragged on until July, when I was asked to see her in the hope that there might be some prospect of operative relief, since she was still suffering from irregular periods of fever (103°–104°) during which there were marked stupor, severe headache, and cervical retraction. She had been ill in all about eight weeks. Though under the impression that there was some mechanical obstruction with hydrops ventriculorum, a lumbar puncture during one of her stuporous periods evacuated a large amount of not particularly turbid fluid which was under high tension. To my astonishment the fluid was swarming with *Diplococcus intracellularis,* both inside and outside of cells. Forty-eight hours later, on the return of the stupor, another puncture was made and *15 c.c. of antimeningitis serum* were introduced into the spinal canal; the diplococci were still numerous. The temperature dropped to normal soon after. After a second forty-eight hours *this was repeated*; diplococci were few. Again in forty-eight hours there was a repetition of the puncture and *serum injection*; practically no diplococci could be found in the fluid, though there may have been a few organisms present. From this time on there were no further symptoms—no headache and no fever. She rapidly convalesced and has recovered her usual health."

Discussion.—The instructive points of this case relate to the persistence of the diplococcus in large numbers in the cerebro-spinal membranes for a number of weeks after the acute stage of the meningitis had passed off, and the action of the lumbar punctures and serum injections in interrupting and finally and quickly abating the free development of the diplococcus. Apparently the number of diplococci present at the second puncture, before any serum was injected, was not remarkably smaller than at the first puncture, while the next two notes describing the punctures and injections of serum dwell particularly on the diminution in numbers of the diplococcus. The subsidence of symptoms and return to perfect health were undoubtedly attendant upon and probably the outcome of the disappearance of the diplococcus from the cerebro-spinal membranes, and this disappearance was greatly aided by, and possibly accomplished through, the withdrawal of infected spinal fluid and the injection of the antiserum.

THE EPIDEMIC OF MENINGITIS IN GREAT BRITAIN.

A severe epidemic of cerebro-spinal meningitis, caused by *Diplococcus intracellularis,* prevailed in several cities in England, Scotland and Ireland during the winter of 1906–7, and is still prevailing in those countries. We have been fortunate in securing the co-operation in testing the antiserum of Dr. Claude Ker, of the City Hospital of Edinburgh, and of Dr. A. Gardner Robb, of the Belfast City Fever Hospital and the Belfast Union Fever Hospital, of Belfast. We have received preliminary reports, based on a small number of cases of meningitis treated with the antiserum, from these gentlemen which are given in this place. They will doubtless publish their experiences in full later, after a larger number of cases, treated with the antiserum, have come under their observation.

Dr. Ker writes under the date of September 17, 1907:

"Our experience with the serum has so far been limited to four cases. The first three have done well and seem now, after four and three weeks' treatment in hospital, to be out of danger. Our general impression here is that, although averagely acute cases, they have done most exceptionally well. One, however, I fear will be permanently deaf.

"The fourth case was fulminant with profuse hæmorrhages in the skin. It was admitted 18 hours from the onset and died about 24 hours from the onset. It was therefore hopeless, but I thought it just and right to give it the chance, and it had one dose of *30 c.c. of the serum,* which could hardly have had time to be effective. The other cases had about 120 c.c. on an average each. The only selection I am making is to treat cases which are less than a week old.

"By the way, my bacteriologist tells me that it is exceedingly difficult to get the diplococci to stain as usual the day after the first intraspinal injection. This has not been noticed so much with other serums. The germs are there of course and can be seen, but they must be modified in some way by the treatment, as they certainly lose their staining power."

Dr. Robb writes under the date of October 23, 1907:

"Since my return to duty on 1st September the opportunities of testing the serum have not been many, but the results in the cases receiving it have been remarkably satisfactory. I have only had four cases admitted to hospital quite early in the attack since that time.[1]

"The first case was a man of 22 who was 48 hours ill when admitted. He was wildly delirious with normal temperature, cyanosis and plentiful petechiæ. I considered his case practically hopeless from my former experience. I gave him

[1] In addition, two chronic cases of meningitis were treated, as appears from the latter part of the letter.

30 c.c. of the serum after drawing off 90 c.c. of turbid fluid in which the meningococcus was present. 36 hours later he was quite conscious and had no headache; his temperature rose to 101° F. and remained about that level for some days; he had very abundant herpes, but his symptoms rapidly improved. I repeated the *30 c.c. injections at intervals of 3 days,* giving 90 c.c. in all. He made a complete recovery. The remarkably sudden clearance of the mental symptoms and the complete disappearance of the headache were very striking, and as this has taken place in other cases treated with the serum, I think the serum must have the credit.

"The second acute case was a girl of 12 years with very severe attack admitted on the second day. This case was very severe, but the prognosis without serum would have been uncertain. The same good results followed with good recovery.

"The third case, a woman of 31, had been ill 10 days with very severe attack; high temperature, delirium, and very marked rigidity. My prognosis would have been bad, but she rapidly improved after the serum and is in a fair way to recovery now. She had 90 c.c.

"The fourth acute case, a man of 20, with maniacal delirium, came in two days ago. I gave him *30 c.c. of serum* immediately on admission—then 46 hours ill. The cerebro-spinal fluid was quite purulent even then. He died of heart failure some 9 hours after receiving the serum.

"Even more striking were the results in two chronic cases. One boy not doing well, who had continuous fever for 35 days, received *30 c.c. of the serum.* From that time on he made steady improvement, had no further headache and has made a complete recovery. Another case which I considered quite hopeless got 30 c.c. on the 25th, and again on the 32d day. He made steady improvement from the first dose and is now up. One young child, who was also hopeless, showed no improvement and died, but in this case thick, stringy pus was obtained. In the chronic cases no improvement had followed simple drawing off of fluid.

"I quite appreciate how dangerous it is to draw conclusions from a few cases, especially during a lull in the epidemic, but allowing for all that I am greatly impressed with the results in the cases I have had. I believe there has been little if any change in the virulence of the type here. The cessation of the headache in the chronic cases receiving serum and in whom it had been most troublesome was very striking; and the absence of headache in the acute cases after serum I have not seen in any other cases."

Discussion.—There could be no advantage gained in discussing these cases, since they are reported so very briefly. Attention may, however, be called properly to the effects of the serum injections in the two cases of the chronic disease described by Dr. Robb. They recall the similar effects of the serum injections made in the chronic case of epidemic meningitis described by Dr. Cushing, of Baltimore.

ST. VINCENT'S HOSPITAL CASES, NEW YORK CITY.

The antiserum was not available for use in the treatment of meningitis during the prevalence of the severe epidemic in New York City. Since it has been available only occasional sporadic cases of the disease have continued to appear, and many of these have entered hospitals late in the course of the disease. Hence our opportunities for testing the antiserum under conditions of personal observation have been very few. We have secured from Dr. Strain, of St. Vincent's Hospital, the records of three cases of meningitis in adults caused by *Diplococcus intracellularis* of which abstracts follow.

CASE I, No history of onset obtained; total serum injection 30 c.c.; death.
CASE II, 1st injection of serum on 4th day; total serum injection 30 c.c.; recovered by lysis.
CASE III, No history of onset obtained; total serum injection, 60 c.c.; recovered by lysis.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE I. A. C., aged 22 years. Greek.

Present Illness.—No history could be obtained. Admitted to hospital, April 23, 1907.

Physical Examination.—The patient is in a semi-comatose condition. Pupils unequal; right pupil does not react to light; left is dilated; no strabismus. Herpes on upper lip. Marked retraction of the head and stiffness and tenderness of neck. Reflexes absent from extremities; Kernig's sign present. Babinski reflex absent.

April 23. Patient is somnolent, pulse slow and of medium tension; general condition poor. Temperature ranged from 102°–100°.

April 24. Patient noisy, crying out. No remarkable change. Leucocytes 18,000.

April 25. General condition about the same. 15 c.c. of turbid spinal fluid obtained by lumbar puncture. Microscopical examination showed pus cells and *Diplococcus intracellularis.*

April 26. Condition worse; the pulse is rising gradually and the first heart sound is becoming weaker; capillary circulation very poor; cyanosis; cold extremities. Temperature 100°–103°; pulse 99–112; respirations 28–34.

April 27. Condition about as yesterday. 20 c.c. of turbid spinal fluid removed by lumbar puncture and *30 c.c. of antimeningitis serum injected.* Following this the temperature rose 1° and then gradually declined during the night to 99°, the patient's condition gradually becoming worse.

April 28. Condition very bad; pulse hardly perceptible; respiration accelerated; physical signs unchanged.

April 29. Lumbar puncture unsuccessful. Condition bad.

May 1. Death at 1 a. m.

Discussion.—The duration of the disease in this case is not known. The diagnosis of epidemic meningitis, which was suggested by the symptoms, was established by lumbar puncture on April 25, two days after admission to hospital. The second puncture and the first injection of the serum were made on April 27, four days after admission to the hospital and at a time when the patient's condition was already very poor and the circulation had begun to fail markedly. No improvement followed the puncture and injection, but the patient's condition gradually grew worse until his death on May 1, eight days after admission to the hospital, four days after the serum injection, and an unknown period after the onset of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE II. C. L. Aged 22 years. Greek. Laborer.

Present Illness.—On May 7 (one day before admission to hospital) the patient was taken suddenly ill with sharp chill and intense headache. The neck became stiff, and slight movement greatly increased pain in head and neck. There were fever and photophobia.

Physical Examination.—Flushed face; anxious expression; injected conjunctivæ; photophobia; beginning herpes on nose and lips; tongue coated; breath fœtid. Neck stiff and head retracted. Kernig's sign marked; reflexes absent; skin hyperæsthesia.

May 8. Temperature 102°, pulse 90, respirations 24 on admission. Patient quiet, but complains of headache. Under ice cap slept much of the day.

May 9. Patient somnolent; cries out at times. Temperature ranged from 103.4° to 105°; pulse 80–94.

May 10. At 9 a. m. and 1 p. m. temperature 104°.—4 p. m., lumbar puncture yielded 35 c.c. of turbid fluid containing pus cells and large numbers of diplococci. Headache better; slept much of night.—5 a. m. Temperature 100°.

May 11, 4 p. m. Lumbar puncture; 30 c.c. spinal fluid withdrawn and *30 c.c. of antiserum injected.* During the night patient was very noisy and required sedatives (morphia and hyoscin) to give sleep.

May 12, 9 a. m. Temperature 104°; remained high all day. No marked change in the condition.

May 13, 5 a. m. Temperature 100°. Pulse slow and full; mental condition apathetic. Temperature did not rise above 101°. Involuntary bladder and bowel movements.

May 14. Condition improved; mental condition brighter. Patient follows with the eyes persons and objects about the room.

May 15. No marked change. Temperature ranged from 101.8° to 103°.

May 16. Condition better. Awake greater part of day; headache less; does not complain of pain in the neck.

May 17. Improvement continues; patient comfortable; mentally brighter; less rigidity of neck; appetite improved.

May 19. Temperature normal; neck rigidity and headache almost gone; patient bright and cheerful.

The improvement in the patient's condition continued and he was discharged cured on June 8.

The leucocyte count on three occasions gave 11,000, 15,000 and 14,000.

Discussion.—The onset of the symptoms in this case was sudden and by the end of the first twenty-four hour period the disease had reached full development. On the third day, counted from the appearance of the first symptoms, lumbar puncture was made and the diagnosis established by bacteriological examination. The temperature fell, the headache diminished, and the patient rested better than before. On the fourth day a second puncture was made and the antiserum was injected. No immediate favorable effect followed, but 60 hours later the condition had improved and there was a steady subsequent inprovement up to complete recovery. Convalescence may be said to have begun not later than the tenth day of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE III. G. P. Aged 18 years. Greek. Clerk.

Present Illness.—No definite history obtainable. About a week before admission to hospital on May 18, 1907, he complained of headache and vomiting.

Physical Examination.—Face flushed and expresses pain; photophobia; pupils equal and react to light; tongue coated and dry; neck rigid and head retracted; a few petechial spots on thorax, abdomen and legs; reflexes exaggerated; no clonus or Babinski; Kernig's sign marked. May 18 the temperature rose from 98.5° to 103°; pulse 85; respirations 28.

May 19–28. The condition has not greatly altered. The mental condition dull, there is much headache and at times delirium requiring restraint. The temperature did not rise above 102.5° and ranged about 101°. Leucocytes 24,000.

May 27. Leucocytes 21,000.

May 29. Patient quiet and somnolent. By lumbar puncture about 50 c.c. of turbid fluid were obtained and *30 c.c. of antiserum injected.* Following the injection the patient was quiet and the temperature fell to 99°. Microscopical examination of spinal fluid showed pus cells and intra- and extracellular diplococci, which were cultivated.

May 30 and 31. Mental condition somewhat improved; no delirium.

June 1. External strabismus appeared, otherwise no change.

June 2. Mental condition clearer; patient answers questions. Temperature fluctuating.

June 4. Patient complains of pain in neck and is at times somewhat delirious. About 50 c.c. of spinal fluid removed by lumbar puncture and *30 c.c. antimeningitis serum injected.* The temperature fell from 103° to normal in twenty-four hours. Delirium subsided and patient looked brighter. He had involuntary defecation and micturition.

June 6. Leucocytes 20,000.

June 7. Patient noisy at night, requiring sedatives.

June 10. Pulse weak but responds to digitalin.

June 11. Neck rigidity lessened; patient fairly comfortable; photophobia and strabismus gone; diet increased; temperature normal.

June 15. Patient gradually getting stronger; mental condition good.

June 18. Improvement continues.

June 21. Patient noisy and delirious all day; temperature 99°. For the next two or three days he was delirious at times.

June 27. Patient rational all day and slept and ate well. Temperature 97°.

June 29. Patient sat up for the first time.

July 5. Walked a few steps; leucocytes 6,000.

July 20. Discharged cured.

Discussion.—It is impossible to determine accurately the duration of the disease before the patient entered the hospital. On the eleventh day after admission lumbar puncture was made and a serum injection was given. Following these the patient's mental condition improved somewhat, but three days later external strabismus appeared. On the seventeenth day of illness a second lumbar puncture and serum injection were made which were followed within twenty-four hours by a marked fall in temperature and an improvement in the patient's mental condition. The temperature remained about normal afterwards, although there reappeared off and on for a few days, during the convalescence, a state of temporary delirium, which did not interrupt the general course of improvements in the patient's condition. Recovery was complete. Whether the serum injections influenced favorably the progress of the disease in this case cannot be determined with certainty. Total amount of serum injection, 60 cubic centimeters.

MANNER OF ACTION AND OF EMPLOYING THE ANTISERUM.

The plan to administer the antiserum by direct inoculation into the spinal canal in human beings was based upon the observations made by one of us (Flexner) on the bactericidal effect of normal sera and sterile exudates upon *Diplococcus intracellularis in vitro,*

and upon the curative action of antidiplococcus sera in guinea-pigs and monkeys infected with the diplococcus, when brought into immediate relation with the focus of infection. In view of certain theoretical objections to the employment as curative agents of antisera developed for a microorganism whose toxic action is caused by endotoxin, Flexner dwelt on the encouraging circumstance that in epidemic meningitis the main pathological lesions can be brought directly under the influence of the antiserum by injecting the latter into the spinal canal; and he pointed out that while it is undoubtedly important to secure neutralization of the endotoxin yielded by the diplococcus on disintegration, the effect of restraint of growth and multiplication of the diplococcus may, at some period of the disease, be of even greater significance.[5] There is experimental evidence for the view that the antiserum possesses a certain antitoxic value since it can neutralize the toxic substances contained in autolysates of the diplococcus. But its power to bring about rapid suppression of the diplococcus in infected guinea-pigs and monkeys is considerable. In monkeys which have been injected with mixtures of emulsions of the diplococcus and immune serum simultaneously, or first with the emulsion and next with the immune serum, the diplococcus is caused rapidly to diminish in numbers and to be more abundantly taken up by leucocytes.[6] Since the facts at hand do not warrant us in concluding that any considerable multiplication of the diplococcus takes place in the experimental infections, the power of protection of the antiserum would appear to be dependent upon the restraint which it exercises over all multiplication and the increased tempo of phagocytic inclusion of the diplococcus which it brings about. It is probable that phagocytic digestion not only prevents further multiplication of the diplococcus but also that it detoxicates the endotoxin by reducing it to simpler and non-toxic or less toxic compounds. Still, in a few instances, in which the antiserum was injected into the spinal canal of monkeys infected with the diplococcus, the microorganisms disappeared without marked phagosis and more slowly than in the cases in which outpouring of

[5] *Jour. of Exper. Med.*, 1907, ix, 138.
[6] *Idem*, p. 169 *et seq.*

leucocytes was considerable. The control of the pathological conditions in these instances appeared to depend less upon the phagocytes than upon the spinal fluid reinforced by the antiserum; and as the symptoms of intoxication were less than would have been present had the antiserum not been injected, a degree of antitoxic power must be ascribed to the antiserum.

If we turn to our knowledge of the manner in which the antiserum acts in controlling or modifying the infection in human beings we find ourselves possessed of very few facts. The observation recorded by Dr. Cushing indicates that the antiserum has the property of bringing about rapid diminution in the number of diplococci present in the cerebro-spinal fluid. Dr. Ker observed rapid rise of the opsonic index for the diplococcus following upon an antiserum injection and a modification and reduction of the staining power of the diplococcus in film preparations prepared from the meningeal exudate obtained by lumbar puncture. Others have noted this reduction in number and change in the staining properties of the diplococcus after the serum injections.

We have had the opportunity to follow in two young children the immediate effects of the antiserum injections on the number, appearances and viability of the diplococcus in the cerebro-spinal fluid. One of the children was 18 months old and had been two weeks ill when first injected at the Babies Hospital in New York. The spinal fluid withdrawn before the serum injection was slightly turbid and showed a fair number of extracellular and a large number of intracellular, sharply-staining diplococci. Cultures were easily secured from this fluid on blood and sheep-serum agar media. A second puncture made twenty-four hours after an antiserum injection yielded a fluid of the same appearance as before, but no extracellular diplococci, or very few, were contained in it, and the number of intracellular diplococci was much reduced. All the diplococci were more or less changed; they were swollen or fragmented and stained diffusely. Cultures were now negative. A second antiserum injection was given and the next day the diplococci had undergone a still greater reduction in numbers and continued to stain feebly. No cultures could be obtained from this fluid or any subsequent fluid from this child, although several later punctures were made.

The second child was two years old and had been ill about five days when admitted to the Presbyterian Hospital in New York under Dr. Northrup. The first lumbar puncture yielded a sero-purulent fluid containing large numbers of diplococci outside and inside pus cells. Abundant cultures mere easily obtained. An injection of 15 cubic centimeters of antiserum was given and twenty-four hours later the lumbar puncture yielded a fluid in which a little blood obscured the color, but the pus cells and diplococci were diminished in numbers. The latter were now almost wholly inside cells and of irregular size and contour and weak staining power. A portion of the fluid was centrifugalized and the sediment used for preparing cultures which did not grow. Serum injection and lumbar puncture were repeated on two later occasions. The spinal fluid withdrawn was far less purulent than it had been, the diplococci became very few in number, and they did not again multiply on culture media otherwise favorably adapted for the growth of the diplococcus.

These observations, few in number as they are, go to show that the antiserum exerts a definite and injurious influence upon the diplococcus in the cerbro-spinal fluid through which its multiplication is restrained and it is rendered more subject, possibly, to phagocytic inclusion and digestion, at the same time that it is deprived of its capacity to grow outside the body on culture media.

We have given the following general instructions for the use of the serum:

The antiserum should be kept in a refrigerator until it is to be used, when it should be warmed to the body temperature before it is injected.

The antiserum is to be introduced directly into the spinal canal after the withdrawal of cerebro-spinal fluid by means of lumbar puncture.

The quantity of antiserum to be used at a single injection should not exceed, for the present, 30 cubic centimeters. It is desirable, although it would not appear to be essential, to withdraw from the spinal canal at least as much fluid as the amount of antiserum to be injected. The injection should be made slowly and carefully to avoid the production of symptoms due to increased pressure. This

precaution should be exercised especially where the quantity of cerebro-spinal fluid withdrawn is less than the amount of antiserum to be injected.

The injection of the antiserum should be repeated every twenty-four hours for three or four days or longer. Whether any advantage will be gained by more frequent or more numerous injections than here indiciated a wider experience must decide. As much as 120 cubic centimeters of the antiserum have been injected into the spinal canal in four days without causing unpleasant symptoms.

The evidence at hand indicates that the earlier in the course of the disease the injections are made the better the results. Hence should the film preparation prepared from the first fluid obtained by spinal puncture show Gram-negative diplococci, some of which are within leucocytes, an injection of the antiserum should be made immediately and without waiting for the result of culture tests. Should the diagnosis be left in doubt or the disease prove later to be of another nature than epidemic meningitis, no harm will have been done by the injection of the antiserum.

Although the best results have thus far been obtained where the antiserum has been injected early in the disease, yet the serum should be used in its later stages also until our knowledge governing the value of the serum becomes more precise. The indications at present are that it is useless to employ the serum in the very late stages of the disease in which chronic hydrocephalus is already developed.

Precise records of the manner of action of the antiserum upon the general symptoms of the disease and the local inflammation and the diplococcus should be kept. Information is greatly desired on the influence of the antiserum upon the number, appearances, growing properties, etc., of the diplococcus, upon the relation of the diplococcus to phagocytosis, and on the number and appearances of the leucocytes, before and after the antiserum injections. Counting the leucocytes in the circulating blood, before and after the injections, would help determine whether the antiserum tends to bring a greater number of leucocytes into the inflamed membranes, or whether it leaves the number unchanged or causes cessation of the emigration.

Until the antiserum is proven to be of value or of no value in the treatment of epidemic meningitis its manner of action should be carefully observed and recorded so that a definite decision may be reached as quickly as possible.

DOES ANAPHALAXIS OCCUR FROM REPEATED INJECTIONS OF THE SERUM?

That the human organism reacts more vigorously to second and subsequent injections of horse serum than to the first injection is shown by the reports of many instances in which these stronger effects were noted after administering diphtheria antitoxin. v. Pirquet and Shick[7] call this condition of greater reaction on the part of the animal organism "serum-disease." Wolf-Eisner[8] sees in this state of intensified effect or hypersensibility the fundamental pathological condition underlying the manner of reaction of the animal body to repeated injections of foreign proteids in general, including the bacterial endotoxines. Our precise knowledge of serum-hypersensibility—or anaphalaxis—is due to the impulse given the study of the subject by Theobald Smith and to the exact studies of Otto,[9] Rosenau and Anderson,[10] Besredka and Steinhardt,[11] Gay and Southard,[12] Lewis[13] and others. The particular fact that concerns us at this moment is whether a possible danger to the patient is to be feared from intraspinal injection at considerable intervals of a foreign serum. We know that the intensified effects in man of repeated serum injections under the skin causes discomfort but does not menace life. Besredka and Steinhardt have, however, shown that it is precisely the direct inoculation of the central nervous system with the alien serum in a hypersensitive guinea-pig that is to be feared. It is, therefore, of the first importance to us to ascertain whether a similar danger exists in relation to the intradural injection of the antimeningitis serum.

[7] Die Serumkrankheit, Vienna, 1905.
[8] *Berl. Klin. Woch.*, 1907, xliv, 38.
[9] *Leuthold-Gedenkschrift*, 1906, i, pt. 1, 153.
[10] *U. S. Marine Hosp. Service Hygiene Lab. Bull.*, 1906, No. 29.
[11] *Annales de l'Institut Pasteur*, 1907, xxi, 117, 384.
[12] *Jour. of Medical Research*, 1907, xi, 143.
[13] *Jour. of Exper. Med.*, this number.

There is no danger, apparently, to be apprehended from a single injection of even a considerable volume of the serum into the spinal canal. Daily intradural injection of the antiserum seem also to be well borne, at least, for several days. The question arises whether it is safe to give the injections at intervals of many days, since an interval is necessary in order that the reaction of hypersensibility shall be developed. Dr. Ladd's Case V, a child of three years, was injected on the following dates: April 1, 6, 22; no ill effects followed and recovery was complete. His Case XV, a child of eight years, was injected as follows: June 16 intradurally, June 18 idem, June 21 subcutaneously, June 25 idem, June 27 idem, July 10 intradurally; no ill effects followed and the child recovered. Still other instances of repeated injection, with intervening long interval between certain injections, will be found among the recorded cases. The danger does not, therefore, seem to be great.

We wish now to refer to an infant who was injected at the Babies Hospital, New York City, several times with the antiserum. The fourth injection was made 42 days after the first and 16 days after the third injection and was followed by convulsions, prolonged rigidity and elevation of temperature.

Babies Hospital, New York. Service of Dr. L. Emmett Holt.

E. F. Female child, 11 months old. About the middle of August developed fever, hyperæsthesia, rigidity and projectile vomiting. Admitted to Babies Hospital September 6, 1907. Lumbar punctures on September 7 and 11: turbid fluid withdrawn. September 12, 45 c.c. fluid withdrawn and *5 c.c. antiserum injected.* September 17, 25 c.c. fluid withdrawn, no injection. September 28, 7 c.c. fluid withdrawn and *7 c.c. antiserum injected.* October 8, 3 c.c. fluid withdrawn and *5 c.c. antiserum injected.* No symptoms developed following these injections. October 24, 60 c.c. fluid were withdrawn and *20 c.c. antiserum injected.* Previous to the last injection the child was relaxed and opisthotonos was absent. She was restless and irritable, vomited occasionally, cried if disturbed, and was apparently deaf. The reflexes were increased and there was marked emaciation. The antiserum was administered intradurally at 11 a. m. At 3 p. m. a severe convulsion occurred and marked hyperextension and opisthotonos developed. These were still present on October 29. The temperature before the injection was about 98 to 99°; for four days after the injection it rose to 102° and once reached 104°.

The conditions which developed in this child following the last injection of serum, after an interval of 16 days since the preceding injection, cannot be explained readily. We prefer to leave the

question open whether the phenomena belong to the anaphalactic state or are of another nature. But so far as the case bears on the general question of the intradural injection of the serum it has theoretical rather than practical significance. The antiserum will, as a rule, discharge its beneficial effects in the first days of its employment and for this no danger is known to exist. Rarely, after a resting period in its use, a relapse of the acute infection may call for another injection. The records of the use of the antiserum in relapses do not show that any ill effects followed the injections. We think that the reinjection of the serum in supposed relapses should be based upon demonstrated reappearance of or increase in the diplococcus, since mere sudden rise in the temperature, in the course of meningitis, may obviously be due to other causes than a reinvasion of *Diplococcus intracellularis.*

MANNER OF PREPARING THE ANTISERUM.

The antimeningitis serum employed in the treatment of the cases of epidemic meningitis described in this paper has been made in the horse. The general method of preparation has been as follows:

The first inoculation consisted of cultures of the diplococcus, heated to 60° C. for 30 minutes, injected under the skin. Many different strains of the diplococcus were combined to prepare this vaccine. The first dose was the equivalent of ¼ surface growth on sheep-serum agar in a test tube. The dose was doubled at each subsequent inoculation, until an amount equal to four test tube growths could be given at 5 to 7 day intervals.

Intravenous inoculation was now substituted for the subcutaneous. Beginning with one oese of living diplococci the dose was progressively increased to 2, 3, 5, etc., oese, then ½, ¾, 1, etc., agar slant cultures, and finally to 1½ bottles (12 oz. Blake) of surface growth. The larger quantities of the culture injected into the vein caused such severe reactions and alarming symptoms that they were discontinued.

Subcutaneous and intravenous injection of an autolysate[14] was now used. The doses, at first 1 cubic centimeter, were later increased to 3 cubic centimeters. The injections were made about one week

[14] *Jour. of Exper. Med.*, 1907, ix, 105.

apart. The intravenous injection of the autolysate was discontinued because of the serious symptoms (increased respiration, weakness, etc.) which resulted from them.

At the present time the subcutaneous tissues are being used exclusively for the inoculations, which are made alternately of living diplococci and autolysate at 7-day intervals. Many different strains of the diplococcus are employed in preparing the living cultures and the autolysate for inoculation. The dose of living diplococcus has been increased to one and one half bottles, and of autolysate to the equivalent of one and one half bottles of the cultures.

The febrile reaction to the subcutaneous inoculations is moderate: The temperature rises to 39° to 39.6°. The animal eats less during the febrile period. The local reaction is much greater. Within a few hours a swelling appears at the site of inoculation and extends widely—from the shoulder to the knees at times. The swelling tends to disappear in a few days or, in those instances in which the larger doses of living diplococcus or autolysate are used, sterile abscesses develop which eventually discharge through the skin.

The antiserum has been titrated by the complement-deviation method devised by Kolle and Wassermann,[15] and tested against the autolysate in guinea-pigs. Neither method appears to be quantitatively accurate. The antiserum used in the treatment of the cases of epidemic meningitis described in this paper came from a horse in process of immunization one year or longer before being used for supplying the serum.

Coincidently with our efforts to produce an antidiplococcus serum for use in human beings suffering from epidemic meningitis, Kolle and Wassermann[16] and Jochmann[17] attempted in Germany to prepare such a serum. Only brief reports of the employment of these sera in the treatment of epidemic meningitis have thus far appeared in print. Wassermann[18] has recently reported the results of the treatment of a series of cases of the disease by subcutaneous injections chiefly of his serum, with results which, on the whole, appear

[15] *Deut. med. Woch.*, 1906, xxxii, 16.
[16] *Ibid.*
[17] *Ibid.*, p. 20.
[18] *Ibid.*, 1907, xxxiii, 1585.

to be favorable to the value of the treatment. Schöne[19] treated a still smaller series of cases with Jochmann's serum, partly by the subcutaneous and partly by the intradural method of injection, with results said to have been beneficial, especially where the injections were made into the spinal canal.

GENERAL DISCUSSION.

In view of the small number of cases of epidemic meningitis upon which this paper is based, it would seem the wisest policy, possibly, to defer all general discussion of the results until such time as a larger series of cases treated with the antiserum having been collected, a searching analysis can be made.

On the other hand it seems desirable to recapitulate, at this time, the salient points brought out by the different reports which have been collected in this paper, it being understood that they do not represent conclusions but merely statements of observed facts or obvious deductions from them.

Thus, for example, the records of cases used in the preparation of this paper show that of 47 cases of epidemic meningitis treated with the antiserum, 34 recovered and 13 died. Expressed in percentages, the recoveries equal 72.3 and the deaths 27.6 per cent. The records also show that of the 13 fatal cases four were either fulminant in type, in which death took place within 24 to 36 hours of the onset, or the patient's condition was so extreme that death occurred in a few hours of the injections of the antiserum. If, therefore, these four cases are subtracted there results a total of 43 cases of meningitis treated with the antiserum of which 34 recovered and 9 died, or 79.9 per cent. recoveries and 20.1 per cent. deaths.

This tabulation takes the cases without respect to their duration at the time the treatment was begun. This is manifestly a severe test, but so long as the whole number of cases is so small no other mode of analysis is likely to be as useful. Should it be desired, however, to know the ratio of recoveries to deaths in cases injected with the antiserum in the first three days of illness, the calculation could be based on 18 cases of infection, exclusive of the ful-

[19] *Die Therapie der Gegenwart*, 1907, xlviii, 52.

minant ones, of which 16 or 88.9 per cent. recovered and 2 or 11.1 per cent. died.

In order that these figures should have any value whatever we are obliged to know with what degree of severity the epidemic prevailed at the time and place at which the antiserum was used. We possess, fortunately, good data concerning this point. Eighteen cases of epidemic meningitis occurred at Castalia within a few weeks. Of these twelve patients died and six recovered. Of those patients who recovered three were injected with the antiserum, and no patient having received the serum died.

The report from Akron covers twenty cases of epidemic meningitis of which nine were not treated with the antiserum: Eight died and one recovered. The remaining eleven cases were treated with the antiserum: three died and eight recovered. The three fatal cases included two of the fulminating variety.

Dr. Robb[20] has given statistics covering 230 cases of epidemic meningitis which arose in Belfast. Of these 162 died making a mortality of 70.43 per cent. As regards the severity of the disease at the time at which the antiserum prepared by us was employed he states in his letter (p. 203): "I believe there has been little if any change in the type here."

The majority of the cases which were treated with the antiserum were in children over five years of age and in adults. Since the mortality is highest among young children a table has been prepared, from Dr. Ladd's report, giving the results of the use of the serum in children under five years of age (p. 185). Of the eight children belonging to this group seven were under three years and one was about five years old. Five of the children recovered and three died. Two of the three fatal cases were injected on the fourth day and one on the twentieth day.

The records of the patients who recovered have been full enough in twenty-five instances to enable us to make out the manner of termination of the disease—whether by lysis or by crisis. We have found that thirteen times the disease terminated by lysis and twelve times by crisis. The accompanying table (Table I) illustrates this point and enables the individual case-histories, upon

[20] *British Medical Journal*, 1907, No. 2443, 1129.

TABLE I.

Dr. Ladd's Cases.

Case No.	Day of Disease. First Injection.	Total c.c. Anti-serum Injected.	Recovered by Lysis.	Recovered by Crisis.	Died.
I.	3	25	+		
II.	12	5		+	
III.	4	30	+		
IV.	5	15			+
V.	2	35		+	
VI.	1	15		+	
VII.	3	20		+	
VIII.	3	45	+		
IX.	2	35			+
X.	1	15		+	
XI.	20	25			+
XII.	4	46			+
XIII.	1	45		+	
XIV.	14	28	+		
XV.	3	53	+		
XVI.	4	41			+
		Akron Cases.			
I.	5	82.5	+		
II.	1	10			+ F
III.	12	20	+		
IV.	2	43.5	+		
V.	7	25		+	
VI	2	22.5			+
VII.	1	15		+	
VIII.	1	35			+ F
IX.	2	105	+		
X.	1	25		+	
XI.	2	22.5		+	
		Pennsylvania Hospital Cases.			
I.	4	15		+	
II.	3	25	+		
III.	4	45		+	+
IV.	11	25			
V.	10	15	+		
		St. Vincent's Hospital Cases.			
I.	?	30			+
II.	4	30	+		
III.	?	60	+		
		Edinburgh Cases. Recovered (no details).			
I.	—7	120	+		
II.	—7	120	+		
III.	—7	120	+		
IV.	1	30			+ F

Belfast Cases.

I.	2	90	+		
II.	2	90	+		
III.	10	90	+		
IV.	2	30			+ F
V.	35	30	+		
VI.	25	60	+		
VII.	?	?			+

Dr. Cushings' Case.

I.	56	45	+		

— = less than. F = Fulminating.

which it is based, to be scrutinized. In a number of cases the abrupt termination of the disease, after acute and violent onset, within forty-eight hours of the injection of the antiserum, was striking and impressive. In some cases the serum was injected as early as twelve hours, and in one case as early as two hours, after the onset of severe symptoms, with prompt arrest of the disease.

Once or twice after abrupt arrest of active symptoms lasting several days, relapses occurred which were as promptly controlled by another injection of serum as was the original infection. It would appear that during the height of an epidemic of meningitis spontaneously abortive cases are of infrequent occurrence.

To discuss, on the basis of so small a series of cases as is here presented, whether the antiserum can be said certainly to influence favorably the temperature, mental condition, and such special symptoms as headache, muscular rigidity, paralyses, etc., seems hardly worth while. Moreover an analysis of the reports having these points in view can be made far better by those who have been in daily contact with the ill from epidemic meningitis and have learned, at first hand, to know its protean aspects and variable course. But, on the other hand, it is patent that the successful cases reported here have, almost without exception, made complete and rapid recoveries. There have been few or no long and tedious periods of convalescence, and in one instance only has a permanent defect—in this instance some imparement of hearing—remained.

Our choice of mode of introducing the antiserum into the body, namely into the spinal canal, should be justified. We were led to this manner of employment by two considerations: First, the

theoretical advantage of bringing the antiserum into direct contact with the focus of infection and inflammation, to support which we possess data based on animal experimentation; and second, the knowledge that elimination of colloids, and of crystalloids even, from the blood stream into the cerebro-spinal fluid is a slow and imperfect process in health and probably in inflamed states of the membranes also. Since there appeared to be no danger from this method of introduction of the serum, provided care was exercised, it was chosen; and the cases recorded in this paper bear testimony to its safety. In a few instances the subcutaneous has been superadded to the intradural injection, but whether any advantage is to be derived from such double injections, greater experience will have to determine. Could the subcutaneous be substituted for the intradural method there would obviously be a gain in convenience and probably in safety of general employment of the serum. We think it improbable that the results would be as good by the subcutaneous as they appear to be by the intradural method.

We possess evidence that the direct effect of the antiserum upon the diplococci present in the exudate in the cerebro-spinal membrane is to cause their rapid degeneration and an arrest of their free multiplication. This must be of some advantage to the patient. We have noted remarkable reduction in number and striking evidences of degeneration and loss of power of growth of the diplococcus, twenty-four hours after an injection of the antiserum. An exudate previously sero-purulent may be converted into a merely turbid exudate by an intradural injection of the serum.

The few cases in which the exudate has been really purulent or fibrino-purulent and present in small quantity, as judged by the few drops which could be secured on lumbar puncture, seemed not to be benefited by the serum injections. Whether such cases are very unpromising will have to be determined by wider experience with the antiserum.

Although we have discussed, briefly, the question of anaphalaxis, the cases in which several injections of serum were made, sometimes with considerable intervals between the injections, tend to show that the danger is not a very real or impending one. It can, we think, be neglected in practice for the present.

It is clear that once we accept the fact of the abortive and critically terminating cases as being caused by the antiserum, the early injection of it is to be sedulously sought. The figures, small as they are, also point to better results from the injections made in the first three days of the illness as compared with those made at a later period. And yet favorable effects have been recorded in cases treated in the fourth, fifth and eighth week of the disease. Basing a tentative deduction on the few facts of our present knowledge, we may suppose that as long as living and multiplying diplococci remain in the membranes and their fluid, the serum may be employed with hope or expectation of useful results. The serum is clearly of no avail in the treatment of symptoms resulting from chronic obstructive lesions of the membranes.

The doses of the serum employed thus far rest on an empirical basis. Whether the larger doses which have been used latterly are more efficient than the smaller ones used at first, can be determined only by a wider experience than we have yet had. In test-tube experiments degree of concentration of serum plays an important part in determining injury and disintegration of the diplococcus, since a high concentration of the serum is more effective than a low one. But the mechanism of test-tube bacteriolysis and of intradural bactericidal effect may be and doubtless are widely different. Our knowledge of the manner of *intra vitam* disposal of the diplococcus after serum injection is very defective; but an important factor is doubtless the intracellular, phagocytic digestion for which we have evidence derived from microscopical examination of the cerebro-spinal exudate. While in active stages of the infection the intracellular diplococci present sharp outlines and appear vigorous, and the extracellular microorganisms are well preserved, after the serum injection the diplococci within cells have lost sharpness of outline, stain indifferently, and strike one as degenerated, and those without cells are much reduced in numbers and staining power. Possibly it is this active and apparently accelerated intracellular digestion which prevents an increase in toxic effects following the serum injections such as might otherwise occur from the more rapidly liberated endotoxin. That the antiserum possesses certain direct antitoxic properties, which also tend to di-

minish the dangers of endotoxin-intoxication, would seem to be indicated by its power to neutralize *intra vitam* the toxic effects of an autolysate of the diplococcus.

In order to avoid unnecessary repetition in preparing this discussion, some of the above propositions have been stated in a manner that might readily convey the impression that we regard it evident and established that the antiserum has proven its usefulness as a therapeutic agent in epidemic meningitis. The facts of our belief, at the present time, are quite otherwise. No one could be less convinced of the final fact of its value than we are. On the other hand, we believe that the data at hand warrant a wider trial of the antiserum, particularly as no other and better means of combating the disease is available. We think, however, that it is unjustifiable to employ the serum indiscriminately and without proper clinical and bacteriological controls. We shall be able, at the Rockefeller Institute, to supply a moderate amount of the antimeningitis serum for use under conditions of control which we shall prescribe.

ADDENDUM.

Since the completion of this paper reports of seventeen additional cases of epidemic meningitis in which the antiserum was employed have been received. The full histories of the cases will be published later, but summaries of them will be given here. Twelve of the cases were treated at the Municipal Hospital, Philadelphia, by Dr. Franklin Royer. A tabulation of them follows:

Case No.	Age of Patient in Years.	Day of First Serum Injection.	Total Amount in c.c. of Serum Injected.	Result.
I.	3	7	180	Died.
II.	4	9	90	Recovered.
III.	18	3	90	Died.
IV.	7	3	140	Recovered.
V.	3	4	220	Died.
VI.	10	2	90	Recovered.
VII.	8	6	30	Recovered.
VIII.	22	?	95	Recovered.
IX.	13	3	150	Recovered.
X.	10	8	90	Died.
XI.	10	4	150	Recovered.
XII.	13	3	120	Recovered.

Two babies, each a year old, were treated with the antiserum at Mt. Sinai Hospital, New York, by Dr. Heiman. One received the first injection of serum on the twenty-third day, 60 cubic centimeters were injected in all; it died. The other was injected on the fifteenth day, received 35 cubic centimeters of the serum and recovered.

Two children were treated with the serum at the Babies Hospital, New York, by Dr. Holt. One is eleven months old, was injected first on the forty-ninth day of illness and will probably die. The other is eighteen months old, was injected first on the twenty-third day of illness and is now convalescent.

An additional case, not included in the first series of cases, was treated at the Akron City Hospital. The child was six years old, was injected first on the sixth day of illness, received in all 160 cubic centimeters of antiserum and recovered.

STOMACH FEEDING IN MICE.[1]

By DR. LEWIS HART MARKS.

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(From the Royal Institute for Experimental Therapeutics, Frankfort a/M. Director, Geheimrat Prof. Dr. P. Ehrlich; Hygienic-Bacteriological Dept., Prof. Dr. M. Neisser.)

To be able to feed mice absolutely quantitatively has long been the desire of many experimentors. Of the many methods which have been devised for this purpose only one, that of Ehrlich[2] (feeding with prepared cakes), still survives. This method depends, however, upon the hunger or eating capacity of the individual mouse. The procedure which we present herewith is both quick and most easily performed. Since the introduction of this method, in this Laboratory under the direction of Professor Max Neisser, nearly a year ago, it has been in constant use for many different purposes, all of which form the subject of following papers, with the exception of the lethal and maximum dose of several drugs, which we present herewith.

Method.—The tube employed (Fig. 1) was made for us by the firm of F. and M. Lautenschläger, of this city. It consists of a silk rubber tube with a syringe needle head, being six and one half centimeters long. The forward end is rounded, as in the human stomach tube. The needle head projects very little into the lumen of the tube. With reasonable care these tubes may be kept for a long time. It is advisable to wash out the lumen with alcohol after using, in order to prevent clogging. In order to pass the tube the mouse is held in the following position. An assistant grasps between the thumb and first finger of the left hand the loose skin as far down over the nose as possible, the thumb being in line with the right ear of the mouse. The tail is now grasped

[1] Received for publication, October 28, 1907.

[2] *Deut. Med. Woch.*, 1891, xvii, 976.

with the right hand, the mouse drawn straight, then the tail is passed and held between the ring and little finger of the left hand (Fig. 2). The assistant now passes closed, with the right hand, a small thin bladed forcep, into the mouth, then allows the forcep to open, which movement forces the jaws apart. Care must be taken to have a forcep which does not open too widely (Fig. 3). The tube is now wet with water, grasped about its middle, by the operator, and passed downwards and a little to the side, until its length is lost in the digestive tract and mouth (Fig. 4). Force even of the slightest nature must never be employed. If the mouse is held correctly, and if the tube is placed correctly, only gentle guidance is necessary. The syringe holding the desired quantity is now quickly inserted into the needle head, and the piston forced down. The syringe is quickly removed, and after a little water or salt solution has been drawn in, again inserted into the needle head, and its contents injected. This last procedure washes out all the remaining fluid in both the syringe and tube, and insures the entrance into the stomach of the exact quantity. The tube is removed by one quick upward movement. Mice have not the power of vomiting and are therefore especially adapted for feeding purposes.

MAXIMUM AND LETHAL DOSES.

General Remarks.—We undertsand by the term "Maximum Dose" the largest dose which failed to kill one, from a great number of mice, between seventeen and twenty-five grams in weight. We do not pretend that it is the medicinal dose, or that it can be borne upon successive occasions at short intervals. However, from our experience with the substances mentioned below and with other substances, we can state that as a general rule, if a mouse bears a given dose, by this method, or by intraperitonel or subcutaneous injection, once it can bear the same quantity upon succeeding days.

Calomel.

Owing to its insolubility the giving of this drug, by this method, is not so satisfactory. We have employed it however in suspension, with the following results:

Lethal Dose	0.005 grm.
Maximum Dose	0.002 grm.

Dilute Hydrochloric Acid.

The finding of the lethal dose of hydrochloric acid was due to the fact that we desired to use it as a solvent for quinine, which follows:

Lethal Dose ..... 0.5 c.c. from a 6 per cent. solution.
Maximum Dose .. 0.5 c.c. from a 5 per cent. solution.

Quinine Hydrochlorate, in 3 Per Cent. Hydrochloric Acid.

Lethal Dose ..... 0.1 grm.
Maximum Dose ..... 0.08 grm.

Iodide of Potassium.

Lethal Dose ..... 0.03 grm.
Maximum Dose ..... 0.01 grm.

Sodium Salicylate.

Lethal Dose ..... 0.035 grm.
Maximum Dose ..... 0.02 grm.

Sodium Sulphite.

Lethal Dose ..... 0.2 grm.
Maximum Dose ..... 0.09 grm.

Sodium Sulphate.

Lethal Dose ..... 0.2 grm.
Maximum Dose ..... 0.08 grm.

Magnesium Sulphate.

Lethal Dose ..... 0.4 grm.
Maximum Dose ..... 0.2 grm.

Antipyrine.

Lethal Dose ..... 0.04 grm.
Maximum Dose ..... 0.01 grm.

Bichloride of Mercury.

Lethal Dose ..... 0.0007 grm.
Maximum Dose ..... 0.0004 grm.

Strychnine Nitrate.

Lethal Dose ..... 0.00005 grm.
Maximum Dose ..... 0.00003 grm.

Atoxyl.

Lethal Dose ..... 0.04 grm.
Maximum Dose ..... 0.02 grm.

Sodium Acetyl-paraamido-phenyl-arsenic Acid (Ehrlich).

Lethal Dose ..... 0.3 grm.
Maximum Dose ..... 0.1 grm.

FIG. 1.

FIG. 2.

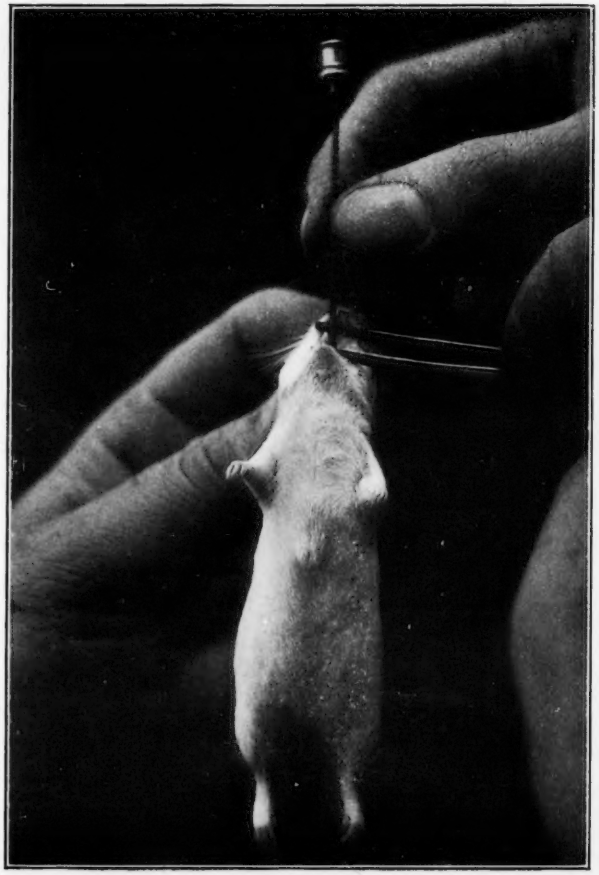

FIG. 3.

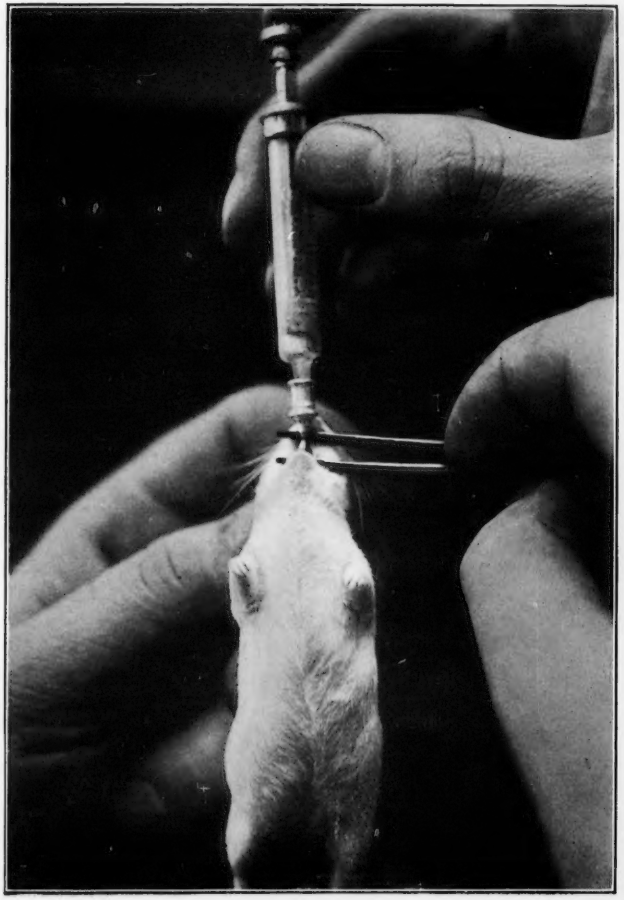

FIG. 4.